ELSEVIER

International Journal of Pharmaceutics 172 (1998) 137–145

international journal of pharmaceutics

# Inulin hydrogels. II. In vitro degradation study

Liesbeth Vervoort *, Patrick Rombaut, Guy Van den Mooter, Patrick Augustijns, Renaat Kinget

*Laboratorium voor Farmacotechnologie en Biofarmacie, Campus Gasthuisberg O + N, Herestraat 49, B-3000, Leuven, Belgium*

Received 29 January 1998; received in revised form 24 April 1998; accepted 19 May 1998

**Abstract**

Inulin hydrogels have been developed as potential new carriers for colonic drug targeting. Since site-specific drug release of this delivery system is based on its bacterial degradation in the colon, the enzymatic digestibility of the prepared inulin hydrogels was assessed by performing an in vitro study using an inulinase preparation derived from *Aspergillus niger*. The amount of fructose liberated from the inulin hydrogels by the action of inulinase was quantified using the anthrone method. The equilibrium swelling ratio as well as the mechanical strength of the hydrogels were studied before and after incubation in inulinase solutions. The data obtained by these different methods indicate that enzymatic digestion of the inulin hydrogels appeared to be enhanced by a prolonged degradation time, a higher inulinase concentration and a lower degree of substitution and feed concentration of the hydrogel polymer. The inulin hydrogels exhibited an increase in equilibrium swelling after degradation compared to the swelling before degradation, suggesting that inulinase enzymes are able to diffuse into the inulin hydrogel networks causing bulk degradation.


*Keywords:* Inulin; Hydrogels; Colon targeting; Inulinase; In vitro degradation; Mechanical strength analysis

## 1. Introduction

Targeting of drugs to the colon is considered to have several therapeutic advantages (Rubinstein, 1990; Ashford and Fell, 1994; Van den Mooter and Kinget, 1995): (1) By treating colonic disorders such as ulcerative colitis, Crohn's disease, colon carcinoma's and infections with a site-specific delivery system, therapy will be more effective, the required dose can be lowered and undesirable side effects can be reduced. (2) For drugs such as peptides and proteins, which are destroyed by the stomach acid and/or metabolised by enzymes of the small intestine, colon targeting can be a valuable alternative for parenteral administration, due to negligible activity of brush-border peptidase activity and much less activity of

* Corresponding author. Tel.: + 32 16 345829; fax: + 32 16 345996; e-mail: liesbeth.vervoort@farm.kuleuven.ac.be

0378-5173/98/$ - see front matter 
*PII* S0378-5173(98)00201-4

pancreatic enzymes in the colon in comparison to the small intestine. (3) colonic release of drugs can also be valuable when a delay in absorption is desired from a therapeutic point of view in the treatment of diseases that have peak symptoms in the early morning, such as nocturnal asthma, angina or arthritis.

Inulin hydrogels have been developed as potential new carriers for colonic drug targeting. Inulin is a naturally occurring polysaccharide (Van Loo et al., 1995) which consists of $\beta$ 2–1 linked D-fructose molecules. Most of the fructose chains have a glucosyl unit at the reducing end (Roberfroid, 1993). Inulin was chosen as candidate polymer for the development of colon-specific hydrogels because the $\beta$ 2–1 osidic bonds are not significantly hydrolysed by enzymes from the endogenous secretion of the human digestive tract (Dysseler and Hoffem, 1995); colonic bacteria however, and more specifically *Bifidobacteria* which constitute up to 25% of the normal gut flora in man (McKellar and Modler, 1989), are able to ferment inulin (Wang and Gibson, 1993; Gibson and Roberfroid, 1995).

In vitro degradation of inulin suspended in Eudragit® RS films was already demonstrated in previous work (Vervoort and Kinget, 1996); in order to prepare inulin hydrogels, the fructose polymer had to be derivatised which can affect its degradation properties. Development of inulin hydrogels has been previously described (Vervoort et al., 1997). Methacryloyl groups were incorporated in the inulin chains by reaction of the fructose polymer with glycidyl methacrylate and aqueous solutions of the resulting methacrylated inulin were subsequently converted into cross-linked hydrogels by free radical polymerisation.

The aim of this study was to investigate the enzymatic degradability of the prepared inulin hydrogels, a prerequisite for their use as colon-specific drug delivery systems. Degradation of the hydrogels in the colon will result in site-specific release of drug entrapped in the polymer network. The in vitro degradation study of the inulin hydrogels was performed by incubating the hydrogels in solutions of inulinase, able to hydrolyse the $\beta$ 2–1 osidic linkages of the inulin chains. With respect to inulin hydrogel degradation, information was acquired on the effect of enzyme concentration, incubation time, degree of substitution and feed concentration of methacrylated inulin.

## 2. Materials and methods

### 2.1. Materials

Chicory inulin (Raftiline HP; average degree of polymerisation between 22 and 25) was kindly provided by Orafti (Tienen, Belgium). *N*,*N*-dimethylformamide, ammonium persulfate (APS), sodium dihydrogen phosphate, dipotassium hydrogen phosphate and citric acid were supplied by UCB (Leuven, Belgium). Glycidyl methacrylate (96%) and isopropanol (anhydrous, 99 + %) were purchased from Acros Organics (Geel, Belgium) and 4-dimethylaminopyridine, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (99%) (TMEDA) and orcinol were obtained from Sigma (St. Louis, MO). Sodium citrate, 1-butanol and D-(−)-fructose were supplied by Merck (Darmstadt, Germany) and anthrone by ICN Biomedicals (OH). Glacial acetic acid was obtained from Riedel-de Haën (Seelze, Germany) and sulfuric acid (concentrated) from Merck Belgolabo (Overijse, Belgium). Novozym 230, an inulinase preparation (1800 U/g) isolated from *Aspergillus niger*, was generously donated by Novo Nordisk (Bagsvaerd, Denmark).

### 2.2. Synthesis of methacrylated inulin

In order to prepare inulin hydrogels, the polysaccharide was first derivatised by incorporating methacryloyl groups in the fructose chains. Synthesis and characterisation of this methacrylated inulin (MA-IN) have been described in a previous paper (Vervoort et al., 1997). Briefly, inulin (50 g) was reacted with glycidyl methacrylate in *N*,*N*-dimethylformamide (200 ml) in the presence of 4-dimethylaminopyridine as catalyst (10 mol.% vs fructose units). By varying the molar ratio of glycidyl methacrylate to inulin, the degree of substitution (DS), i.e. the amount of methacryloyl groups incorporated per 100 fructose units,

could be tuned. After a reaction time of 72 h at room temperature, MA-IN was precipitated and washed in isopropanol, dissolved in Milli-Q water and dialysed for 10 days at 4°C against the same solvent. MA-IN was eventually recovered by lyophilisation. MA-IN with five different degrees of substitution was prepared: DS = 4.4; 8.1; 12.1; 15.4 and 22.3.

### 2.3. Preparation of MA-IN hydrogels

Cross-linked inulin hydrogels were obtained by free radical polymerisation of aqueous MA-IN solutions using APS and TMEDA as radical generating compounds. MA-IN solutions of 16, 22 and 27% w/w were prepared in 0.5 M phosphate buffer pH 6.5. After adding 17.5 $\mu$mol/ml APS and 39.4 $\mu$mol/ml TMEDA buffer, the mixture was divided over molds and free radical polymerisation took place at room temperature for 2.5 h, resulting in cross-linked hydrogels with a diameter of 10 mm and a height of 2–3 mm. After polymerisation, the hydrogels were removed from the molds and washed in demineralised water to remove unreacted MA-IN and initiating compounds. After washing, the hydrogels were dried at room temperature till constant weight.

### 2.4. In vitro degradation study

Dry hydrogels of known weight ($W_d$) were allowed to swell to equilibrium in demineralised water at 37°C and the weight of the swollen hydrogels ($W_s$) was recorded after blotting surface water with tissue paper. The swollen hydrogels were subsequently equilibrated at the same temperature in 15 ml of 0.025 M citrate buffer pH 4.7 (pH optimum of inulinase) after which Novozym 230 was added and incubation started while gently shaking the solutions on a rocking platform (50 strokes/min) (Stuart, UK). After an incubation period of 25, 48, 72 or 168 h, the hydrogels were recovered, washed in ethanol and demineralised water to inactivate and remove the enzyme, and allowed to swell again to equilibrium in demineralised water at 37°C. After recording $W_s$, the hydrogels were dried at room temperature till constant weight ($W_d$). The equilibrium swelling ratio ($q_{eq}$) was calculated before and after incubation according to:

$$q_{eq} = W_s/W_d \quad (1)$$

In addition to the determination of equilibrium swelling before and after degradation, the incubation media were also assayed for the presence of liberated fructose, considered as a measure for hydrogel degradation, by means of the anthrone method (White et al., 1954; Summerfield et al., 1993). The anthrone reagent was prepared by dissolving 50 mg of anthrone in a mixture of 28 ml water and 72 ml concentrated sulphuric acid. 2.5 ml of this freshly prepared reagent was added to appropriate quantities of collected samples of the incubation media. The solution was mixed immediately and incubated for 10 min at 100°C followed by cooling to room temperature. Absorbance of the samples was measured spectrophotometrically (HP 8452A diode array spectrophotometer, Hewlett Packard, Santa Clara, CA) at 625 nm. Four solutions containing 0.08–0.2 mg/ml of fructose were analysed each time together with the samples for the calibration curve. (The same absorption is given by a sugar compound as if it was first hydrolysed and then determined; equal amounts of glucose and fructose give identical absorption values (Morris, 1948)). Corrections were made for the colour contribution of the enzyme. Samples spiked with known amounts of fructose and inulin were also analysed to

Table 1
Validation of the anthrone method (mean ± S.D., $n = 3$)

| | Spiked concentration ($\mu$g/ml) | Determined % |
|---|---|---|
| Fructose | 57.6 | 97.1 (±1.5) |
| | 84.3 | 97.0 (±3.8) |
| Inulin | 59.9 | 100.5 (±0.6) |
| | 87.5 | 100.5 (±2.6) |

validate this method. Table 1 reports the obtained results expressed as percentage of the theoretical values.

### 2.5. Mechanical strength analysis of MA-IN hydrogels

The mechanical strength of the inulin hydrogels, which can be considered as a measure of their integrity, was investigated before and after incubation in inulinase solutions by determining the required force (*g*) for compressing inulin hydrogels per mm distance. A TA-XT 2 universal texture analyser (Wilten Fysika, Berchem, Belgium) was used in the compression mode with a probe of 10 mm diameter. The probe was lowered at a rate of 0.1 mm/s over a maximum distance of 1.5 mm, and the corresponding compression force was measured and recorded. The experiments were performed in a temperature controlled environment at 37°C, and the compression probe and hydrogel support were covered with water proof sandpaper to avoid slippage of the sample during measurement. The hydrogels tested were allowed to swell to equilibrium in demineralised water at 37°C, with or without prior inulinase incubation for 25 or 168 h.

### 2.6. TLC analysis of MA-IN incubation media

In order to investigate whether the enzymatic degradability of MA-IN molecules was affected by its degree of substitution, a TLC analysis of MA-IN incubation media was performed. MA-IN solutions (1% w/w) were prepared in citrate buffer pH 4.7 and incubated with 1.64 U of Novozym 230/ml at 37°C. Blank solutions (no inulinase) were treated similarly. After an incubation period of 24 h, the incubation media were analysed by thin layer chromatography on silica gel 60 (Merck, Darmstadt, Germany) using 1-butanol:acetic acid:water (3:3:2 v/v) as mobile phase. Sugars were detected after spraying with orcinol (10 mg/ml of 50% v/v sulfuric acid), followed by heating for 15 min at 100°C (Imamura et al., 1994). A fructose solution was used as reference.

## 3. Results and discussion

### 3.1. In vitro degradation study

In vitro degradation of inulin hydrogels was studied by incubation with Novozym 230, a commercial preparation containing endo- and exo-inulinase (obtained from *Aspergillus niger*) which can hydrolyse the $\beta$ 2–1 fructosidic linkages of inulin, resulting in the release of free fructose. Degradation was monitored as a function of time by determining the percentage of free fructose vs the initial dry hydrogel weight (DS 8.1; feed concentration, 22% w/w) generated by various inulinase concentrations (Fig. 1). After an initial approximately linear increase, the amount of liberated fructose per time unit gradually levelled off. Degradation rates (% liberated fructose vs initial dry hydrogel weight/h) were calculated from the slope of the initial linear part of the curves and are listed in Table 2. As might have been expected, increased inulinase concentration resulted in an increased degradation rate.

In addition, the equilibrium swelling ratio of the hydrogels was determined before and after degradation as it has been reported (Brøndsted and Kopecek, 1992) that bulk degradation which implies that the enzymes are able to diffuse into the hydrogels, results in an increase in swelling after degradation. The data of Table 2 indicate that the equilibrium swelling of the hydrogels indeed increased after degradation. It may thus be assumed that inulinase can diffuse into these networks; after hydrolysis of the fructose backbone in the hydrogel network, the network density is reduced resulting in less elastic retractive forces and thus increased swelling. The increase in swelling was more pronounced for higher inulinase concentrations.

In order to investigate whether the hydrogel composition (varying degree of substitution and feed concentration of MA-IN) had an effect on the enzymatic degradability, inulin hydrogels were incubated in an inulinase solution (3.34 U/ml) for 25 h and the equilibrium swelling ratio was determined before and after this incubation period. A time interval of 25 h was chosen since the average colonic transit time varies between 20 and 30 h

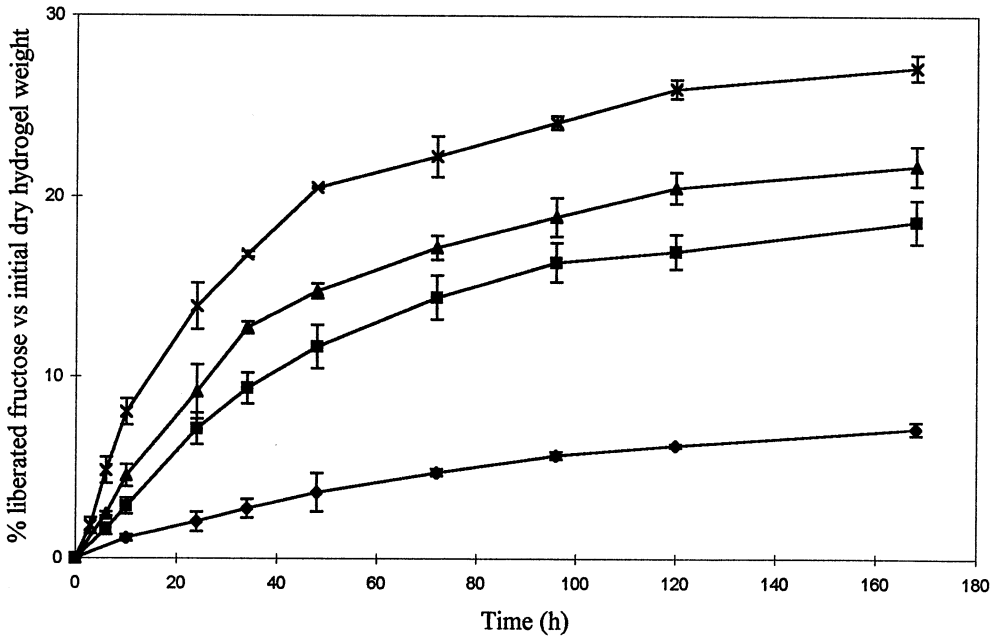


Fig. 1. Cumulative percentage liberated fructose vs initial dry hydrogel weight (DS = 8.1; feed concentration, 22% w/w) as a function of time for various inulinase concentrations (◆ = 0.17 U/ml, ■ = 0.84 U/ml, ▲ = 1.67 U/ml and × = 3.34 U/ml) (mean ± S.D., $n = 3$).

(Van den Mooter and Kinget, 1995). Table 3 indicates that hydrogels prepared from higher substituted MA-IN exhibited less increase in swelling after incubation. The limited bulk degradation could be caused by a decreased permeability of these networks to inulinase since hydrogels with increasing degree of substitution of MA-IN were characterised by a restricted equilibrium swelling, attributed to an increasing intermolecular cross-link density (Vervoort et al., 1998). For the highly cross-linked hydrogels, surface degradation, which does not affect swelling, is probably more pronounced than bulk degradation. Determination of liberated fructose in the incubation media after 25 h revealed that the enzymatic degradation of these hydrogels was anyway lim-

Table 2
Degradation rate (percentage liberated fructose vs initial dry hydrogel weight/h) and percentage increase in equilibrium swelling ratio ($q_{eq}$) after a degradation period of 7 days for hydrogels prepared from 22% w/w solutions of MA-IN DS 8.1 as a function of inulinase concentration (mean ± S.D., $n = 3$)

| Inulinase concentration (U/ml) | Degradation rate | % increase in $q_{eq}$ |
|---|---|---|
| 0.17 | 0.084 (±0.031) | 9.3 (±1.1) |
| 0.84 | 0.31 (±0.03) | 18.5 (±0.5) |
| 1.67 | 0.36 (±0.10) | 29.7 (±3.3) |
| 3.34 | 0.89 (±0.04) | 36.2 (±3.1) |

Table 3
Percentage increase in equilibrium swelling ratio ($q_{eq}$) and percentage liberated fructose vs initial dry hydrogel weight for hydrogels of different composition after a degradation period of 25 h (inulinase: 3.34 U/ml) (mean ± S.D., $n = 3$)

| Hydrogel composition (DS; feed concentration % w/w) | % increase in $q_{eq}$ | % liberated fructose |
|---|---|---|
| 4.4; 27 | 182.4 (±17.6) | 44.9 (±18.8) |
| 8.1; 27 | 7.4 (±1.2) | |
| 12.1; 16 | 18.7 (±0.8) | 18.0 (±3.6) |
| 12.1; 22 | 8.8 (±0.9) | |
| 12.1; 27 | 2.4 (±0.7) | 1.8 (±0.1) |
| 15.4; 27 | 2.0 (±0.3) | |
| 22.3; 27 | 0.5 (±0.2) | 0.9 (±0.1) |

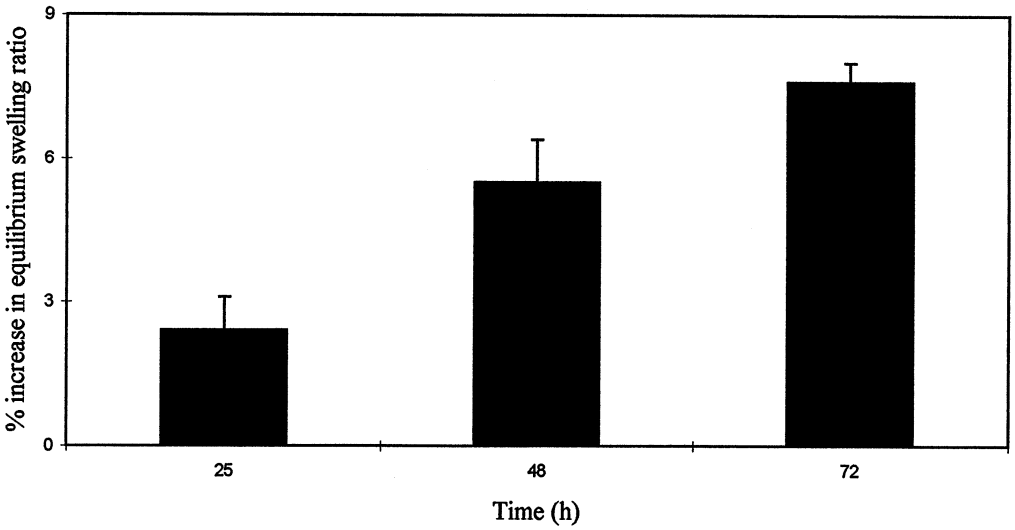


Fig. 2. Percentage increase in equilibrium swelling ratio as a function of incubation time (inulinase: 3.34 U/ml) for hydrogels prepared from 27% w/w solutions of MA-IN DS 12.1 (mean ± S.D., $n = 3$).

ited (Table 3). A possible explanation for this observation is a decreased accessibility of MA-IN to inulinase or a decreased affinity of inulinase for MA-IN with increasing degree of substitution (Shalaby and Park, 1990). This hypothesis was verified by TLC analysis of MA-IN solutions after 24 h of incubation with inulinase at 37°C. In contrast to the blank solutions, fructose ($R_f =$ 0.58, compared to the reference solution) was detected in all incubation media, irrespective of the degree of substitution of MA-IN. This illustrates that despite derivatisation MA-IN can still be degraded by inulinase. However, in contrast to native inulin which was completely degraded to fructose, MA-IN degradation was incomplete, since oligomers ($R_f < 0.58$) could be detected in the degradation media. Increasing amounts of oligomers (based on a semiquantitative estimation of the stains on the TLC plate) were associated with an increasing degree of substitution of MA-IN, indicating that indeed less sites were available for inulinase activity with increasing degree of substitution.

In addition to the degree of substitution, the feed concentration of MA-IN also influenced the enzymatic digestibility of the inulin hydrogels (Table 3). As dilution is considered to promote intramolecular cross-linking or loop formation (James and Guth, 1947; Yeh et al., 1995) and consequently reduces the hydrogel intermolecular cross-link density, hydrogels of low feed concentration exhibit a higher equilibrium swelling ratio (Vervoort et al., 1998). The more pronounced degradation (higher percentage increase in $q_{eq}$ after incubation) of these hydrogels, in comparison with hydrogels of high feed concentration, can thus be attributed to a higher permeability of these networks to inulinase.

The influence of incubation time on percentage increase in $q_{eq}$ for hydrogels prepared from 27% w/w solutions of MA-IN DS 12.1, is illustrated in Fig. 2. When the incubation time in the inulinase solution was prolonged, the hydrogels exhibited a higher increase in equilibrium swelling. This confirms the observations depicted in Fig. 1.

### *3.2. Mechanical strength analysis of MA-IN hydrogels*

Equilibrium swollen hydrogels of different composition were compressed with constant rate before and after enzymatic degradation. The linear part of the obtained force-displacement curves was used to calculate the required force ($g$) per mm compression which was considered to be an

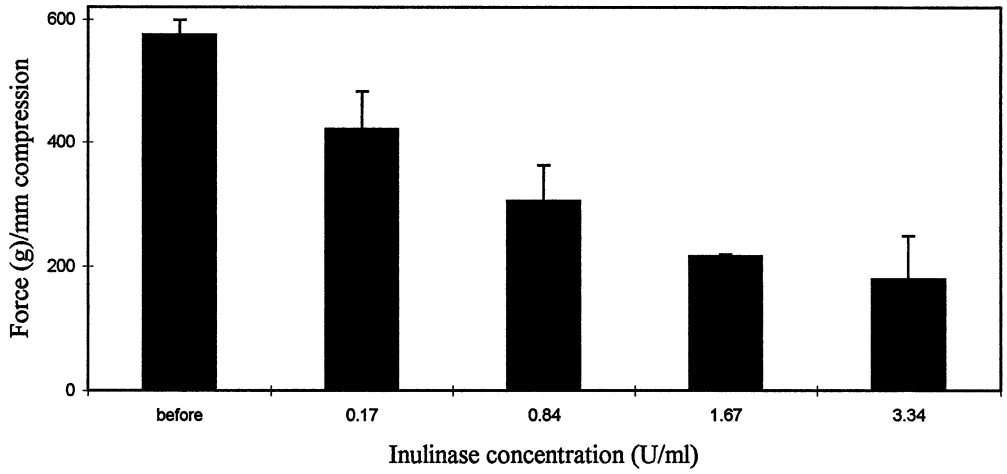


Fig. 3. Required force ($g$) per mm compression for hydrogels prepared from 22% w/w solutions of MA-IN DS 8.1 before and after an incubation period of 7 days in inulinase solutions of various concentration (mean ± S.D., $n = 3$).

indication for the mechanical strength and the integrity of the hydrogel networks. A higher intermolecular cross-link density results in an increased mechanical strength and consequently an increased resistance to the lowering of the compression probe. Enzymatic degradation of the hydrogels causes a loss of network integrity by hydrolysis of the hydrogel backbone. This results in a decreased resistance to compression.

Fig. 3 represents the force ($g$) per mm compression values for hydrogels (DS 8.1; feed concentration, 22% w/w) before and after incubation in Novozym 230 solutions of various concentrations (these hydrogels had already been used for monitoring enzymatic degradation as a function of time resulting in an incubation period of 7 days in this part of the study). The data clearly indicate a reduction of hydrogel resistance to compression, and thus reduced network integrity, with increasing inulinase concentration. The loss of hydrogel integrity after degradation indicates that bulk degradation occurred which supports the results of the swelling experiments.

Compression data for hydrogels of different composition, before and after a 25 h incubation period (3.34 U/ml inulinase), are listed in Table 4. (For hydrogels of 27% w/w solutions of MA-IN DS 4.4, no value could be obtained after degradation because these hydrogels were too weak to manipulate.) However, no significant difference (two tailed, unpaired $t$-test; $p < 0.05$) in mechanical strength before and after degradation could be detected, irrespective of the feed composition of the hydrogels tested. The sensitivity of this method does probably not allow detection of limited changes in hydrogel integrity.

With respect to the mechanical strength of the hydrogels tested before degradation, Table 4 clearly illustrates that higher substituted MA-IN or more concentrated MA-IN solutions resulted in stronger hydrogels. This indicates that these hydrogels are characterised by a high intermolecular cross-link density, which fully agrees with previously obtained results (Vervoort et al., 1998): hydrogels prepared from MA-IN with a high degree of substitution or feed concentration were characterised by a low equilibrium swelling; this observation was attributed to a high intermolecular cross-link density which restricts network expansion upon swelling.

## 4. Conclusion

The data provided in this study indicate that despite derivatisation and cross-linking, inulin hydrogels can still be enzymatically degraded by inulinase. As expected, degradation was depen-

Table 4
Required force ($g$) per mm compression for hydrogels of different composition, before and after a degradation period of 25 h (inulinase: 3.34 U/ml) (mean ± S.D., $n = 3$)

| Hydrogel composition (DS; feed concentration % w/w) | Force ($g$) per mm compression before degradation | Force ($g$) per mm compression after degradation |
|---|---|---|
| 4.4; 27 | 112 (± 30) | — |
| 8.1; 27 | 1338 (± 346) | 1007 (± 165) |
| 12.1; 16 | 480 (± 61) | 367 (± 2) |
| 12.1; 27 | 1893 (± 142) | 1831 (± 104) |
| 15.4; 27 | 2800 (± 107) | 2776 (± 318) |
| 22.3; 27 | 3453 (± 284) | 3503 (± 129) |

dent on the incubation time and enzyme concentration. The enzymatic digestion of the inulin hydrogels also appeared to be dependent on the permeability of the hydrogel networks to inulinase: an increasing degree of substitution or feed concentration of MA-IN resulted in stronger hydrogels, attributed to an increased intermolecular cross-link density, and hence, diffusion of enzymes into these dense hydrogel networks is hampered, limiting bulk degradation (less increase in equilibrium swelling after degradation). In addition to the permeability of the networks, the accessibility of MA-IN to inulinase and the affinity of inulinase for MA-IN, which can both be influenced by the degree of substitution, also have to be considered with respect to hydrogel degradation.

## Acknowledgements

We thank Orafti (Tienen, Belgium) for the generous gift of Raftiline HP, Novo Nordisk for donating Novozym 230 and Professor W.E. Hennink for co-operation concerning initiation in the derivatisation method of inulin.

## References

Ashford, M., Fell, J.T., 1994. Targeting drugs to the colon: delivery systems for oral administration. J. Drug Target. 2, 241–258.

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

Dysseler, P., Hoffem, D., 1995. Inulin, an alternative dietary fiber. Properties and quantitative analysis. Eur. J. Clin. Nutr. 49, S145–S152.

Gibson, G.R., Roberfroid, M.B., 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. 125, 1401–1414.

Imamura, L., Hisamitsu, K., Kobashi, K., 1994. Purification and characterisation of $\beta$-fructofuranosidase from Bifidobacterium infantis. Biol. Pharm. Bull. 17, 596–602.

James, H.M., Guth, E., 1947. Theory of the increase in rigidity of rubbers during cure. J. Chem. Phys. 15, 669–683.

McKellar, R.C., Modler, H.W., 1989. Metabolism of fructooligosaccharides by *Bifidobacterium* spp. Appl. Microbiol. Biotechnol. 31, 537–541.

Morris, D.L., 1948. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science 107, 254–255.

Roberfroid, M.B., 1993. Dietary fiber, inulin, and oligofructose: a review comparing their physiological effects. Crit. Rev. Food Sci. Nutr. 33, 103–148.

Rubinstein, A., 1990. Microbially controlled drug delivery to the colon. Biopharm. Drug Dispos. 11, 465–475.

Shalaby, W.S.W., Park, K., 1990. Biochemical and mechanical characterisation of enzyme-digestible hydrogels. Pharm. Res. 7, 816–823.

Summerfield, A.L., Hortin, G.L., Smith, C.H., Wilhite, T.R., Landt, M., 1993. Automated enzymatic analysis of inulin. Clin. Chem. 39, 2333–2337.

Van den Mooter, G., Kinget, R., 1995. Oral colon-specific drug delivery: a review. Drug Delivery 2, 81–93.

Van Loo, J., Coussement, P., De Leenheer, L., Hoebregs, H., Smits, G., 1995. On the presence of inulin and oligofructose as natural ingredients in the western diet. Crit. Rev. Food Sci. Nutr. 36, 525–552.

Vervoort, L., Kinget, R., 1996. In vitro degradation by colonic bacteria of inulin HP incorporated in Eudragit RS films. Int. J. Pharm. 129, 185–190.

Vervoort, L., Van den Mooter, G., Augustijns, P., Busson, R., Toppet, S., Kinget, R., 1997. Inulin hydrogels as carriers for colonic drug targeting. I. Synthesis and characterization of methacrylated inulin, and hydrogel formation. Pharm. Res. 14, 1730–1737.

Vervoort, L., Van den Mooter, G., Augustijns, P., Kinget, R., 1998. Inulin hydrogels. I. Dynamic and equilibrium swelling properties. Int. J. Pharm., 172, 127–135.

Wang, X., Gibson, G.R., 1993. Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. J. Appl. Bacteriol. 75, 373–380.

White R.P., Samson F.E., Kan L., 1954. Determination of inulin in plasma and urine by use of anthrone. J. Lab. Clin. Med. 475–478.

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