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Research paper

# Colonic delivery of compression coated nisin tablets using pectin/HPMC polymer mixture

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**Abstract**

Nisin containing pectin/HPMC compression coated tablets were prepared and their in vitro behavior tested for colonic delivery. Nisin is a 34-amino-acid residue long, heat stable peptide belonging to the group A lantibiotics with wide antimicrobial activity against Gram-positive bacteria. The invention can be useful for treating colonic infectious diseases such as by *Clostridium difficile*, and also by colonization of vancomycin-resistant *enterococci*. In this study, each 100 mg core tablet of nisin was compression coated with 100% pectin, 90% pectin–10% HPMC, 85% pectin–15% HPMC, 80% pectin–20% HPMC, 75% pectin–25% HPMC, 100% HPMC at a coat weight of 400 mg. The concentration and the activity of nisin were quantified using Well Diffusion Agar Assay. Drug release studies were carried out in pH 3.3 buffer solution. System degradation/erosion experiments were carried out in pH 1.2, 3.3, and 6.8 buffers using a pectinolytic enzyme. The biological activity and NMR studies were performed to assess the stability of nisin during the processing and after the in vitro tests. It was found that pectin alone was not sufficient to protect the nisin containing core tablets. At the end of the 6 h 40% degradation was observed for 100% pectin tablets. HPMC addition required to control the solubility of pectin, a 5% increase in HPMC ratio in pectin/HPMC mixture provided a 2-h lag time for nisin release. Eighty percent pectin–20% HPMC appeared to be an optimum combination for further evaluation. Tablets maintained their integrity during the 6-h dissolution test, approximating the colon arrival times. Nisin was found to be active/stable during processing and after in vitro tests. Effect of polymer hydration on pectin degradation was found to be crucial for the enzyme activity. Sufficiently hydrated pectin degraded faster. The pectin/HPMC envelope was found to be a good delivery system for nisin to be delivered to the colon.


*Keywords:* Colonic delivery; Nisin; Pectin; HPMC; Compression coating; NMR; Well Diffusion Agar Assay

## 1. Introduction

Colonic drug delivery has gained increased importance not just for the delivery of drugs for the treatment of local diseases of colon such as irritable bowel syndrome, inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis but also for its potential for the delivery of proteins and therapeutic peptides like insulin [1,2]. Colon as a site offers distinct advantages on account of a near neutral pH, a much longer transit time, reduced digestive enzymatic activity, much greater response to absorption enhancers, and the presence of large amounts of enzymes for polysaccharides (e.g., β-D-glucosidase, β-D-galactosidase, amylase, pectinase, xylase, dextranase, etc.) which were secreted by a large number and variety of colonic bacteria [3,4]. Various systems have been developed for colon-specific drug delivery. These include covalent linkage of a drug with a carrier, coating with pH-sensitive polymers, time dependent release systems, and enzymatically controlled delivery systems [5]. Enteric coated systems are the most commonly used for colonic drug delivery, but the disadvantage of this system is that the pH difference between small intestine and colon is not being very pronounced. These delivery systems do

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not allow reproducible drug release. The limitation of time dependent release system is that it is not able to sense any variation in the upper gastro-intestinal tract transit time, any variation in gastric emptying time may lead to drug release in small intestine before arrival to colon. Apparently, the most convenient approach for site-specific drug delivery to colon is enzymatically controlled delivery systems. No drug release can occur unless the system arrives to the colon [1–6].

To improve the specificity of drug release, certain types of neutral polysaccharides (e.g., pectin, chitosan, dextran, guar gum, inulin) can be used to create the enzymatically controlled delivery systems. Many protein and peptide drugs cannot be administered through the oral route because of their degradation by the digestive enzymes of upper gastro-intestinal tract. To reach the colon and to be able to specifically deliver a peptide or a protein drug, the dosage form must be formulated taking into account the obstacles of upper gastro-intestinal tract and advantages of colonic environment [2,7,8]. Nisin is a naturally occurring, ribosomally synthesized protein and was discovered in 1928. It is produced by the Gram-positive bacterium *Lactococcus lactis subsp. lactis*. It shows antimicrobial activity against a wide range of Gram-positive bacteria, such as food borne pathogens *Listeria monocytogenes*, *Clostridium botulinum*, *Staphylococcus aureus*, and their spores [9–11]. Nisin contain dehydro residues [dehydroalanine (DHA) and dehydrobutyrine (DHB)] and thio-ether cross-linkages (lanthionine and β-methyllanthionine) that are introduced by post-translational modifications of ordinary amino acids (serine, threonine, and cysteine). For the antimicrobial activity the integrity of nisin must be maintained (Fig. 1). It is now proposed that the antimicrobial effect of nisin is due to the incorporation of nisin to the cytoplasmic membrane and pore formation in it, leading to membrane disruption and efflux of cytoplasmic substances (e.g., $K^+$, ATP, and amino acids). It also inhibits spore germination [12,13]. Nisin is used as a natural preservative and has been applied in food preservation and dental care products [14]. Digestive enzymes rapidly inactivate nisin and consequently it does not alter the bacterial microflora in the intestinal tract. The U.S. Food and Drug Administration affirmed nisin as *GRAS* for use as a direct ingredient in human food [15]. It has also been reported as non-toxic ($LD_{50} = 7$ g/kg) [16,17]. Nisin solubility and stability increase substantially with increasing acidity. Its optimum stability between 3 and 3.3 and can be autoclaved at 121 °C. Nisin activity is usually quantified using Well Diffusion Agar Assay. The assay sensitivity for nisin was greatly increased by using *Lactobacillus sakei ATCC 15521* as the indicator organism [18–21]. The activity of nisin is expressed in terms of international units (IU), and 1 g of pure nisin is equivalent to $40 \times 10^6$ IU, while 1 g of Nisaplin®, a commercial nisin reference (Applin & Barrett, UK), is equivalent to $1 \times 10^6$ IU [22,23].

In this study, it was the first time nisin was used as a model for a peptide drug. Nisin was tableted as the core, and then compression coated with pectin/HPMC mixture to form an enzymatically controlled delivery system.

## 2. Materials and methods

### 2.1. Materials

Nisin (Nisaplin®), de Man Rogosa and Sharpe agar and broth (MRS Agar, MRS Broth), Noble Agar were kindly donated by Applin & Barrett (United Kingdom), Oxoid (United Kingdom), and Difco (United Kingdom), respectively. Nisin sensitive indicator organism *L. sakei ATCC 15521* DSMZ (Germany) was used in "Well Diffusion Agar Assay". Pectin USP 100 was obtained from Copenhagen Pectin (Denmark), Hydroxypropyl methyl cellulose HPMC (Metolose SR, Type 90sh, 100,000 cP), pectinex ULTRA SP-L (26,000 FDU/ml) was a gift from Shin-Etsu Chemicals Ltd. (Japan), and Novo-Nordisk Ferment Ltd. (Switzerland), respectively. All other chemicals were, analytical grade and stock culture was maintained at −80 °C in MRS Broth with 20% glycerol.

### 2.2. Methods

#### 2.2.1. Nisin standards

A stock solution of nisin was prepared adding 250 mg of Nisaplin® to 25 ml of 0.02 N HCl + 0.75% NaCl (pH

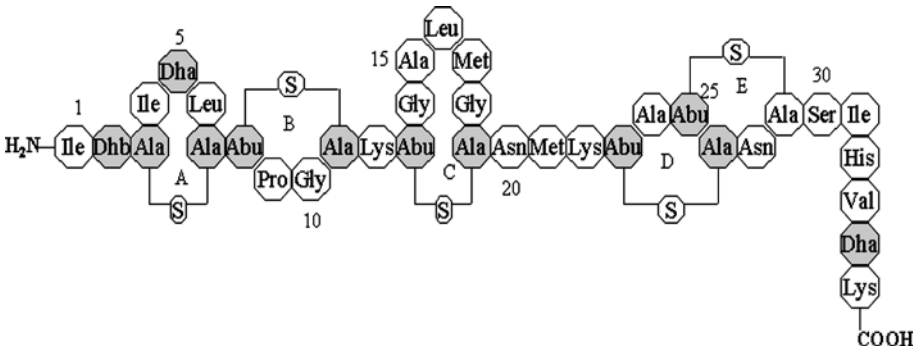



Fig. 1. Primary structure of nisin. Abu, amino butyric acid; Dha, dehydroalanine; Dhb, dehydrobutyrine; Ala-s-Ala, lanthionine; Abu-s-Ala, β-methyllanthionine (Shang-Te D. Hsu et al. 2004) [32].

3–3.3). The stock solution was then filtered through a 0.22 μm filter. Nisaplin® contains by definition, $10^6$ IU of nisin per gram, making the stock solution 10,000 IU/ml. Dilutions (1000, 800, 600, 400, 200, 100, 80, 60, 40, 20 IU/ml) were prepared by mixing nisin stock solution with sterile 0.02 N HCl + 0.75% NaCl (pH 3–3.3).

### 2.2.2. Comparison of preincubated and non-preincubated methods

*Lactobacillus sakei ATCC 15521* stock culture was maintained at −80 °C in 20% glycerol. *L. sakei ATCC 15521* was maintained on MRS Agar at 4 °C and grown one night in MRS broth and incubated at 30 °C prior to incorporation into the media for nisin assay. The cell suspension in MRS broth was centrifuged at 4000*g* for 20 min at room temperature. The cells, subsided in tube, were taken into 0.9% sterile salt solution and adjusted McFarland 0.5 standard for blurriness ($1 \times 10^8$ cfu/ml). The molten agar was tempered at 45 °C and seeded with 1.25 ml aliquot of the suspension of indicator organism to final density of approximately $0.5 \times 10^6$ cfu per ml. Each 20 ml of inoculated medium (MRS broth + 1.5% Noble Agar) was dispensed to petri plates (9 mm diameter). After the agar solidified, 6.6 mm wells were cut with a sterile metal borer. Fifty microliters of nisin standard was pipetted into the well and the plates were preincubated at 4 °C for 24 h. Then incubated at 30 °C in some cases the plates were directly incubated at 30 °C to see the effect of preincubation. After the incubation, inhibition zones were measured horizontally and vertically using a caliper (Inox, SOMET) and well diameter was subtracted from the result and averaged. Diameters of *inhibition zones vs* $log_{10}$ *nisin concentrations* were plotted in order to obtain a standard curve and comparison of preincubated and non-preincubated methods. Method linearity, precision, accuracy, and selectivity were studied to validate the method.

### 2.2.3. Preparation of nisin core tablets

Nisaplin® (commercial nisin = $10^6$ IU/g), lactose, mannitol and PVP K30 were mixed with each other according to the geometrical dilution method. Water was added to granulate. Wet granules were sieved through 1 mm screen and dried 5 h at 40 °C. After adding 1% stearic acid as a lubricant tablets weighing 100 mg each were compressed using a laboratory size single station tablet press (Korsch EKO) with 6 mm flat faced punches. Tablet quality control tests such as weight variation, crushing strength, friability, thickness, and dissolution were performed on the core tablets.

### 2.2.4. NMR studies of powder and compacted nisin

Since nisin has a polypeptide structure H NMR spectrums of both nisin powder and directly compressed nisin tablets were studied to see whether it protected the structural integrity or not. All samples were dissolved in DMSO and H NMR spectrums were run at BRUKER Avance 500 NMR device. Using H NMR spectrum the probability of breaking down of polypeptide chain under compaction was investigated.

### 2.2.5. In vitro release studies of nisin core tablets

Nisin core tablets were placed in vessels of Sotax A7 dissolution tester and 250 ml of 0.02 N HCl + 0.75 NaCl (pH 3–3.3) solution was added as the release medium. Dissolution studies were performed using apparatus II of the USP 24 at 50 rpm/37 °C. At various time intervals, a sample of 5 ml was withdrawn and replaced with equal volume of fresh medium. Withdrawn samples were filtered through a 0.22 μm filter and 50 μl of sample was pipetted into the well of solidified agar seeded *L. sakei ATCC 1552.* Plates were preincubated at 4 °C/24 h and then incubated 30 °C /24 h. After the incubation, the inhibition zone was measured and % nisin release was calculated.

### 2.2.6. Compression coating of core tablets

Nisin core tablets were placed in 10 mm die cavity of a laboratory hydraulic press. Depending on the design, 100% pectin, 90% pectin–10% HPMC, 85% pectin–15% HPMC, 80% pectin–20% HPMC, 75% pectin–25% HPMC, and 100% HPMC combinations were used for the outer shell compression coating. Coating pressure was 200 MPa and the coating weight was 400 mg. Tablet quality control tests such as weight variation, crushing strength, friability, thickness, dissolution/erosion rates in different media were performed on the compression coated tablets.

### 2.2.7. Pectin/HPMC coat erosion studies in 0.1 N HCl and pH 6.8 USP Buffer solution

After compressing the nisin containing tablets an erosion study was performed. First medium was 500 ml of 0.1 N HCl solution. The USP 24 dissolution apparatus II was used at 50 rpm at 37 °C (Sotax A7). The test was continued for 2 h, at the end of the time period the medium was discarded and refilled with USP pH 6.8 buffer solution and the test was continued for additional 8 h. At the sixth hour, 3 ml pectinex ULTRA SP-L was added to the dissolution vessels and the test was continued until for a predetermined time. Tablets were dried overnight at 40 °C in an oven and the remaining tablet mass was determined gravimetrically.

### 2.2.8. In vitro release and dissolution/erosion studies of pectin/HPMC compression coated nisin tablets

Pectin/HPMC compression coated nisin tablets were placed in vessels of Sotax A7 dissolution tester and 250 ml of 0.02 N HCl + 0.75% NaCl solution was added as the release medium. Dissolution studies were performed using apparatus II of the USP 24 at 50 rpm at 37 °C. In the sixth hour 3 ml of pectinex ULTRA SP-L was added into each dissolution vessel. At various time intervals a sample of 5 ml was withdrawn and replaced with the equal volume of fresh medium. Withdrawn samples were filtered through a 0.22 μm filter and 50 μl of sample was pipetted into the well of solidified agar seeded *L. sakei ATCC 1552*. Plates

were preincubated at 4 °C/24 h, and then incubated 30 °C/24 h. After the incubation, inhibition zone was measured. Dissolution/erosion studies were performed as in vitro release studies, but this time at various time interval tablets were taken from the vessels, dried at 40 °C/24 h, and weighed.

*2.2.9. Hydration of polymer coating before enzyme addition*

Hundred percent pectin compression coated nisin tablets were placed in vessels of Sotax A7, 500 ml of pH 6.8 USP buffer was added in each vessel. In one set, 5 ml of pectinex ULTRA SP-L was added at the beginning of dissolution study and in the other, 5 ml of pectinex was added at the sixth hour. At a predetermined time intervals tablets were taken from the vessel, dried overnight at 40 °C in an oven and the remaining tablet mass was determined gravimetrically.

*2.2.10. Data analysis*

In order to characterize the mechanism of drug release from the polymer system, the data were fitted to the following semi-empirical mathematical model [$m_t/m_\infty = k \cdot t^n$] which was developed by Peppas and Korshmeyer [24]. Parameters were calculated using LABFIT Curve Fitting Software 7.2.35. by W.C.P. Silva [25]. The "*n*" values were obtained and interpreted whether the release of nisin fits the *fickian* release or not.

## 3. Results and discussion

### *3.1. Selection of Well Diffusion Agar Assay*

In our study, to make a core tablet a pressure of at least 200 MPa was applied to nisin powder. For the antimicrobial activity, nisin has to maintain its structural integrity. If any fracture occurs in the polypeptide chain, no activity is seen from nisin any longer. In the activity test live organisms have to be used. Therefore, Well Diffusion Agar Assay was the right choice for our study. The size of the inhibition zone as presented in Fig. 2 was linear to the log of nisin concentration. The line in the figure was generated from linear regression and had an $R^2$ value of 0.9982. This linearity agrees with the results of Trammer et al., 1964 and Rogers & Montville, 1991 [18,19].

### *3.2. Results of comparison of preincubated and non-preincubated methods*

The preincubation of plates for 24 h at 4 °C was allowed for nisin diffusion while growth of the indicator organism was delayed. It can be seen from Fig. 3 preincubation increased assay sensitivity by increasing zone diameter. Also preincubation increased assay reproducibility by revealing a lesser amount of variability between readings (Tables 1–3). Results obtained from diffusion agar assay complied with the results from Roger & Montville, 1991 and Pongtharangkul et al., 2004 [19,20].

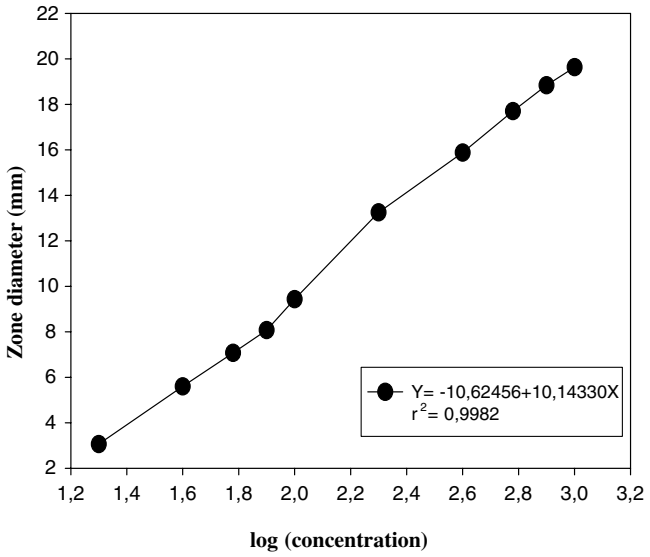



Fig. 2. Standard curve for nisin in 0.02 N HCl + 0.75% NaCl solution.

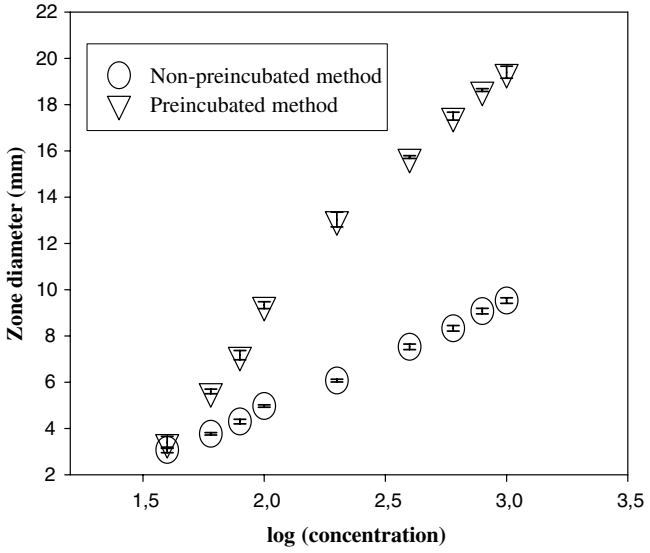



Fig. 3. Comparison of preincubated and non-preincubated methods.

### *3.3. Results of tablet characteristics*

Nisaplin® powder was tried to be compressed directly into a core tablet, but due to its stickiness it could not be achieved. To produce reasonable core tablets it was decided to apply wet granulation. Nisaplin, lactose, mannitol, and PVP K30 were processed together. The physical properties of nisin core tablets and pectin/HPMC compression coated tablets are given in Table 4. It was found that crushing strength of compression coated tablets was dependent on pectin/HPMC polymer ratio. When HPMC ratio in polymer mixture increased the crushing strength of coated tablets increased. HPMC provides mechanical strength to

Table 1
Linearity results for the standard curve ($n = 9$)

| Concentration (μg/ml) | log(concentration) | Average zone diameter (mm) | RSD (%) |
|---|---|---|---|
| 20 | 1.30 | 3.06 ± 0.03 | 1.08 |
| 40 | 1.60 | 5.60 ± 0.05 | 0.82 |
| 60 | 1.78 | 7.07 ± 0.07 | 0.93 |
| 80 | 1.90 | 8.07 ± 0.08 | 1.03 |
| 100 | 2.00 | 9.43 ± 0.07 | 0.70 |
| 200 | 2.30 | 13.25 ± 0.08 | 0.60 |
| 400 | 2.60 | 15.88 ± 0.04 | 0.27 |
| 600 | 2.78 | 17.70 ± 0.08 | 0.45 |
| 800 | 2.90 | 18.84 ± 0.10 | 0.53 |
| 1000 | 3.00 | 19.63 ± 0.07 | 0.39 |

Table 2
Accuracy results of microbiological method ($n = 3$)

| Concentration (IU/ml) | Recovery (%) | RSD (%) |
|---|---|---|
| 50 | 101.32 ± 1.08 | 1.07 |
| 100 | 100.37 ± 0.52 | 0.52 |
| 200 | 99.85 ± 0.87 | 0.87 |

the pectin tablets. All tablets complied with the pharmaceutical quality control standards.

### 3.4. Dissolution results of nisin core tablets

The core tablets containing 40 mg Nisaplin® were tested in 0.02 N HCl + 0.75% NaCl (pH 3.0–3.5) solution for their dissolution rates. Nisin solubility and stability increases substantially with increasing acidity. It has been shown that optimum stability for nisin is at pH 3.0–3.3 [26]. In our study, we decided to follow the assay variables of Rogers & Montville, 1991 [19], by this way we could avoid the unpredictable conditions for the study. Fig. 4 shows the dissolution results of nisin core tablets. The core tablets dissolved in 0.02 N HCl + 0.75% NaCl and reached 100% in 60 min.

### 3.5. H NMR results of powdered and compacted nisin

Results obtained from H NMR spectrum showed that there was no difference between two spectrums. This means no chain fracture occurred during tabletting process. Powdered nisin and compacted nisin had the same spectrum. Lantibiotics contain thio-ether cross-linkages (lanthionine and β-methyllanthionine) that are introduced by post-translational modifications of ordinary amino acids (serine, threonine, and cysteine). These cross-linkages give to the molecule heterodetic pentacyclic structure [9,27]. From the

Table 3
Repeatability and reproducibility results of microbiological method

| Concentration (μg/ml) | log(concentration) | I. day | | II. day | | III. day | |
|---|---|---|---|---|---|---|---|
| | | Average zone diameter ± SD (mm) | RSD (%) | Average zone diameter ±SD (mm) | RSD (%) | Average zone diameter ±SD (mm) | RSD (%) |
| 20 | 1.30 | 3.10 ± 0.03 | 1.00 | 3.14 ± 0.04 | 1.27 | 2.96 ± 0.03 | 1.04 |
| 40 | 1.60 | 5.70 ± 0.05 | 0.90 | 5.50 ± 0.04 | 0.73 | 5.60 ± 0.05 | 0.89 |
| 60 | 1.78 | 7.02 ± 0.07 | 1.00 | 7.13 ± 0.07 | 1.00 | 7.06 ± 0.04 | 0.56 |
| 80 | 1.90 | 8.19 ± 0.09 | 1.00 | 8.01 ± 0.08 | 1.00 | 7.97 ± 0.08 | 1.00 |
| 100 | 2.00 | 9.33 ± 0.04 | 0.43 | 9.45 ± 0.11 | 1.16 | 9.52 ± 0.05 | 0.52 |
| 200 | 2.30 | 13.03 ± 0.03 | 0.23 | 12.95 ± 0.12 | 0.92 | 13.78 ± 0.09 | 0.65 |
| 400 | 2.60 | 15.73 ± 0.03 | 0.19 | 15.91 ± 0.09 | 0.56 | 16.02 ± 0.10 | 0.62 |
| 600 | 2.78 | 17.50 ± 0.07 | 0.40 | 17.79 ± 0.08 | 0.44 | 17.82 ± 0.09 | 0.50 |
| 800 | 2.90 | 18.63 ± 0.08 | 0.43 | 19.01 ± 0.10 | 0.53 | 18.90 ± 0.12 | 0.63 |
| 1000 | 3.00 | 19.40 ± 0.05 | 0.26 | 19.67 ± 0.13 | 0.66 | 19.83 ± 0.05 | 0.25 |

$n = 3$ for all average zone diameter and RSD (%) results.

Table 4
Physical properties of nisin core and pectin/HPMC compression coated tablets

| Code | Weight ± SD (mg) | Crushing strength ± SD (kg.f) | Friability (%) | Thickness ± SD (mm) | Pectin/HPMC ratio (%) |
|---|---|---|---|---|---|
| Core | 99.87 ± 1.33 | 9.84 ± 0.50 | 0.08 | 2.42 ± 0.03 | |
| F1 | 502.9 ± 1.2 | 27 ± 2 | 0.07 | 4.94 ± 0.03 | 100–0 |
| F2 | 503.1 ± 1.5 | 34 ± 2 | 0.05 | 4.74 ± 0.04 | 90–10 |
| F3 | 502.6 ± 0.9 | 37 ± 1 | 0.04 | 4.62 ± 0.02 | 85–15 |
| F4 | 500.8 ± 0.6 | 43 ± 1 | 0.03 | 4.60 ± 0.07 | 80–20 |
| F5 | 501.5 ± 1.1 | 48 ± 1 | 0.03 | 4.52 ± 0.05 | 75–25 |
| F6 | 503.9 ± 1.3 | 55 ± 1 | 0.02 | 4.45 ± 0.03 | 0–100 |

Coating pressure: 200 MPa.
Coating weight: 400 mg.

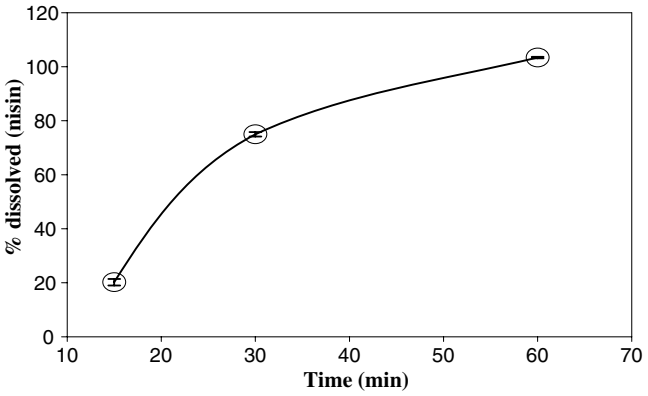



Fig. 4. Dissolution profiles of nisin core tablets in 0.02 N HCl + 0.75% NaCl (USP Apparatus II and 50 rpm).

spectrum results (Fig. 5), we assumed this pentacyclic structure provides structural rigidity on the peptide against heat and pressure. The antimicrobial activity of compacted tablets was also tested on *L. sakei* and no activity loss occurred.

### 3.6. Pectin/HPMC coat erosion studies

The system was designed based on the gastro-intestinal transit time concept under the assumption of colon arrival time of 6 h [28,29]. In this study, we used a high molecular weight HPMC to enforce the mechanical resistance of the tablet during its transit in the GI tract and to partially modify the high solubility of pectin. Pectin/HPMC coat ratios are shown in Table 4. In our previous study (Turkoglu & Ugurlu, 2002) [30], three polymer ratios were studied. In this study, it was decided to enlarge the scale of pectin/HPMC polymer ratios to see how a small ratio of polymer change can affect the polymer erosion. Turkoglu, et al.

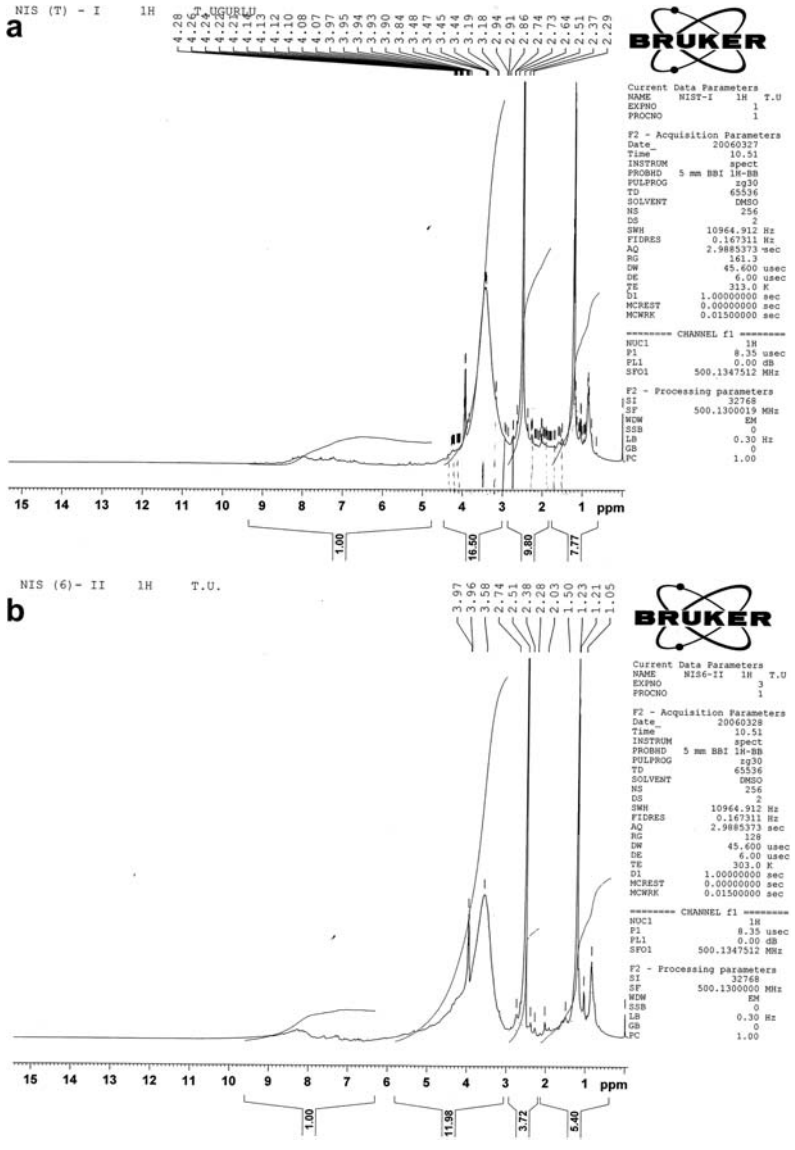



Fig. 5. H NMR spectrum of nisin powder (a) and tableted granule (b).

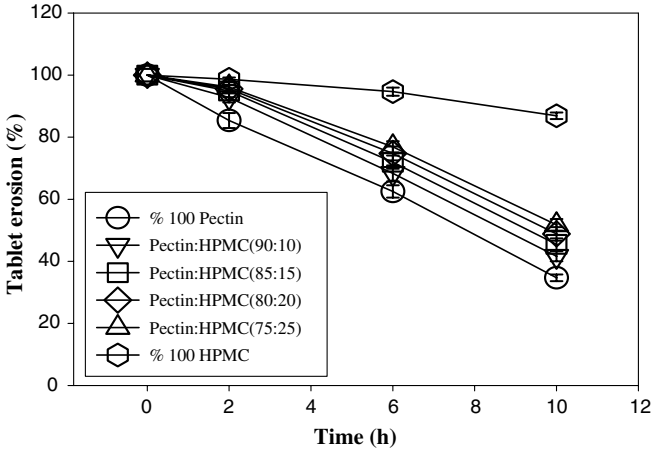



Fig. 6. Percent erosion vs time plot of compression coated nisin tablets ((100:0), (90:10), (85:15), (80:20), (75:25), (0:100) pectin/HPMC polymer mixture). Studies were carried out in 0.1 N HCl and pH 6.8 USP buffer solution. Without pectinex ULTRA SP-L addition, ($n = 6$).

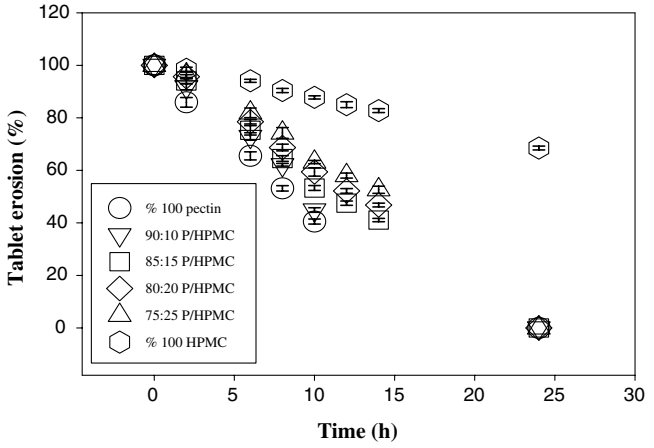



Fig. 8. Percent erosion vs time plot of compression coated nisin tablets ((100:0), (90:10), (85:15), (80:20), (75:25), (0:100) pectin/HPMC polymer mixture). Studies were carried out in 0.02 N HCl + 0.75% NaCl solution. With pectinex ULTRA SP-L addition at 6 h ($n = 6$).

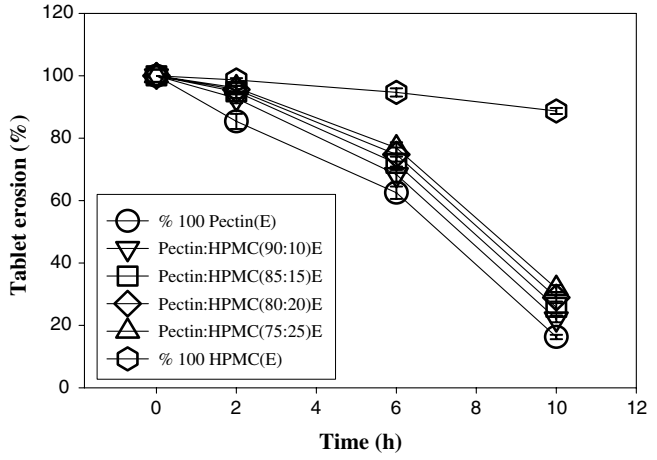



Fig. 7. Percent erosion vs time plot of compression coated nisin tablets ((100:0), (90:10), (85:15), (80:20), (75:25), (0:100) pectin/HPMC polymer mixture). Studies were carried out in 0.1 N HCl and pH 6.8 USP buffer solution. With pectinex ULTRA SP-L addition at 6 h ($n = 6$).

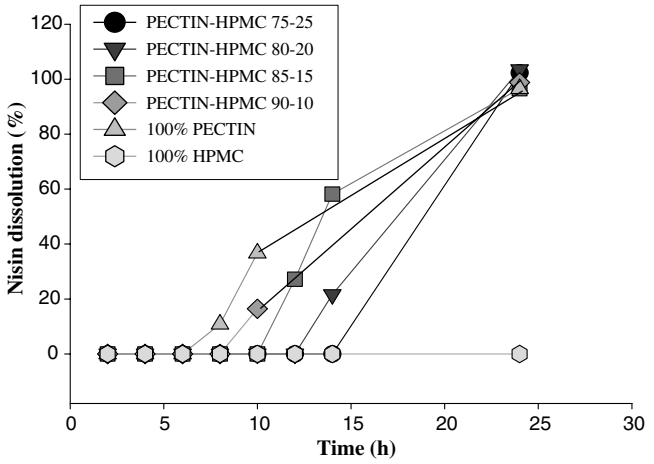
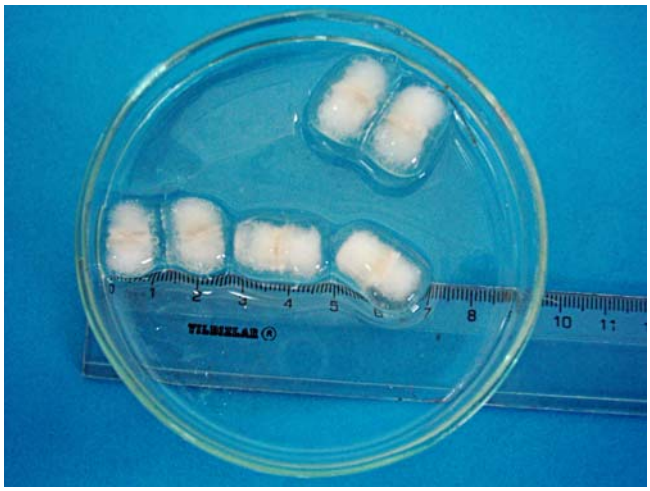



Fig. 9. Nisin dissolution profiles from (100:0), (90:10), (85:15), (80:20), (75:25), and (0:100) pectin/HPMC compression coated tablets. Studies were carried out in 0.02 N HCl + 0.75% NaCl ($n = 6$ and pectinex ULTRA SP-L addition at 6 h).

(1999) [31], showed that pectin dissolved slower in acidic pH than alkaline pH. To mimic GI tract conditions compression coated nisin tablets were kept 2 h in 0.1 N HCl and further 8 h in pH 6.8 USP buffer solution. To determine the effect of pectinolytic enzyme, the erosion studies were carried out with and without enzyme. As seen from Figs. 6 and 7, percent tablet remaining changes in the range of 88–98% and 65–80% at the end of 2 and 6 h respectively. In the absence of enzyme (Fig. 6), there is a linearity in the curves of the diagram. This means constant dissolution of polymer and diffusion of nisin occurred from hydrophilic polymer. In the presence of the enzyme (Fig. 7), biphasic curves occurred. This means dissolution rate changed, due to increased destruction of pectin chains, hence, increasing erosion rate. A 25–30% more tablet dissolution was observed in the presence of enzyme. With the increase in HPMC ratio in polymer mixture, erosion rates decreased.

### 3.7. The results of in vitro release and dissolution/erosion of pectin/HPMC compression coated nisin tablets

0.02 N HCl + 0.75% NaCl solution was selected due to the reasons which were discussed in Section 3.4. Pectin/HPMC system releases the drug enzyme dependently rather than pH dependently, so that, the pH of the medium does not affect the drug release. Pectin and HPMC are hydrophilic materials. The system made from a mixture of these polymers swells and forms a hydrogel layer when they are placed in an aqueous medium. With the diffusion of medium into the polymer a hydrogel layer forms. When there is an enzyme in the environment, it breaks out the polymer



Picture 1. 100% HPMC tablets maintained their integrity during the entire dissolution study. Nisin core tablets can be seen as yellowish lines in the middle of the coat. (For interpretation of the references to colour in this picture legend, the reader is referred to the web version of this article.)

Table 5
The "$n$" constant of formulation according to the Peppas and Korsmeyer equation [24]

| Formulations | Diffusion parameter "$n$" ($m_t/m_\infty = k \cdot t^n$) |
|---|---|
| 100% pectin | 1.61 |
| (90:10) pectin/HPMC | 1.25 |
| (85:15) pectin/HPMC | 1.20 |
| (80:20) pectin/HPMC | 1.37 |

The $n$ value of (75:25) pectin/HPMC tablets could not be calculated due to their nisin release at only in one time point. The $n$ value of (0:100) pectin/HPMC tablets could not be calculated due to their no nisin release during the experiment.

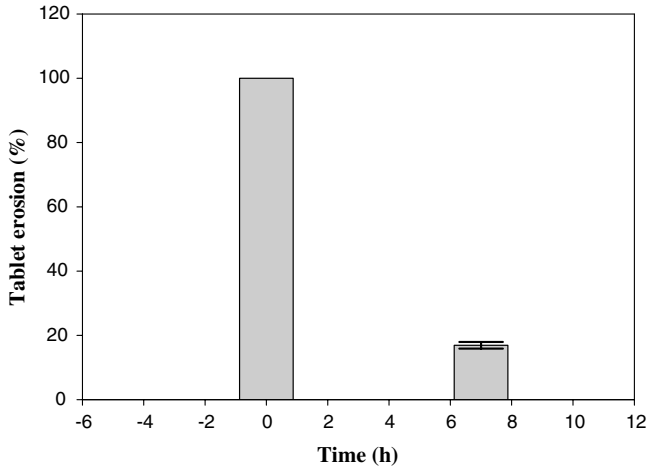



Fig. 10. Percent erosion vs time plot of 100% pectin compression coated nisin tablets. Studies were carried out in pH 6.8 USP buffer solution. Pectinex ULTRA SP-L was added at the beginning of the dissolution/erosion study ($n = 6$).

chains and as a result dissolution of tablet increases with the increase of diffusion of dissolution medium. As the ratio of HPMC increased in the polymer mixture, release

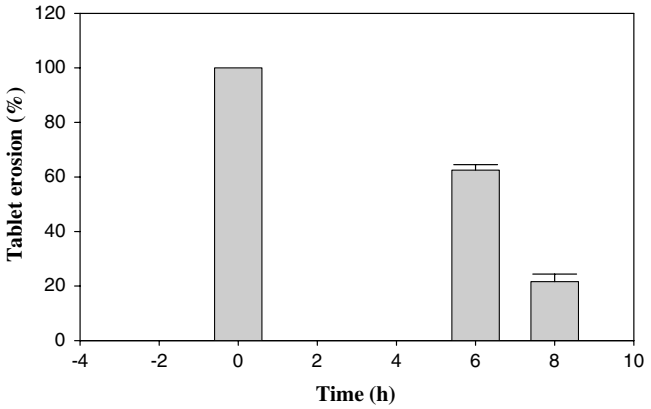



Fig. 11. Percent erosion vs time plot of 100% pectin compression coated nisin tablets. Studies were carried out in pH 6.8 USP buffer solution. Pectinex ULTRA SP-L was added at the 6 h of the dissolution/erosion study ($n = 6$).

of nisin and dissolution/erosion rate of tablets decreased (Figs. 8 and 9). A five percent increase of HPMC in the polymer mixture caused a 2-h lag time for nisin. 100% HPMC compression coated nisin tablets did not release nisin during predetermined time of the dissolution study (Fig. 9 and Picture 1). The "$n$" values demonstrated (Table 5) that pectin/HPMC polymer system shows non-fickian release due to erosion. Based on the studied pectin/HPMC combinations and their erosion/dissolution values, the (80:20) combination was found to be the most promising formula for further evaluation. The (80:20) combination had appropriate mechanical strength and also had a desired erosion profile based on a 6 h colon arrival time concept.

### 3.8. Effect of polymer hydration on pectin degradation

The hydration of polymer mixture was found to be crucial for the enzyme activity. When we added the enzyme at the beginning of the test, all enzyme molecules surrounded the pectin/HPMC polymer coat and this does not allow hydration. If polymer does not hydrate it will not swell. As a result, diffusion of enzyme molecules would be inhibited. However, with the addition of enzyme at the sixth hour, already swollen polymer allows the enzyme to diffuse into the system. Enzyme molecules can easily reach the polymer chains and break them off. Fig. 10 shows the case where the enzyme was added at time = 0 and it took about 6 h for 80% degradation. Whereas, in Fig. 11, the addition of enzyme at the sixth hour acted much faster to degrade pectin due to hydration.

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