Mesoporous Matrices for the Delivery of the Broad Spectrum Bacteriocin, Nisin A

James Flynn, Sarah Mallen, Edel Durack, Paula M. O’Connor and Sarah P. Hudson*

*Department of Chemical Sciences, Synthesis and Solid State Pharmaceutical Centre, Bernal Institute, University of Limerick, Castletroy, Co. Limerick, Ireland.

Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland

APC Microbiome Institute, University College Cork, Co. Cork, Ireland

Corresponding author: Dr. Sarah Hudson, Department of Chemical Sciences and Bernal Institute - sarah.hudson@ul.ie

Keywords: Mesoporous matrices; Nisin A; Controlled release; Antimicrobial; periodic mesoporous organosilanes; simulated gastrointestinal fluid; bacteriocins

Abstract
Mesoporous matrices of different pore size and chemical composition were explored as potential delivery matrices for the broad spectrum bacteriocin, nisin A. The adsorption of nisin A onto two mesoporous silicates (MPS - SBA-15, MCM-41) and two periodic mesoporous organosilanes (PMO - MSE, PMO-PA) was examined. It was found that hydrophobic interactions dominated in the adsorption of this peptide to the matrices, lending the highest adsorption to MCM-41 with a small pore size of 2.8 nm. The hydrophobic ethylene-bridged MSE (6 nm pore) improved the loading and protection of nisin A from degradation by a non-specific protease pepsin, over un-functionalised SBA-15 which had a slightly larger pore size and less hydrophobic moieties. Nisin A did not adsorb onto an amine-functionalised PMO. Upon suspension in modified fasted state simulated gastric fluid (pH 1.6), the highest release of nisin A was observed from MCM-41, with a lower release from SBA-15 and MSE, with release following Higuchi release kinetics. No release was detected into modified fasted state simulated intestinal fluid (pH 6.5) but despite this, the suspended matrices loaded with nisin A remained active against Staphylococcus aureus.

1.0 - Introduction
The current antimicrobial resistance (AMR) crisis has been fuelled by the overuse or misuse of antibiotics thereby exposing pathogenic bacteria to sub-inhibitory levels of antibiotics, inducing resistance. The rise in AMR has also been attributed to the prevalence of antibiotic use in agriculture and household products [1]. The prevalence of resistance in strains such as Streptococcus pneumoniae, Mycobacterium tuberculosis, methicillin-resistant Staphylococcus aureus (MRSA) and
multidrug-resistant Gram-negative bacteria has resulted in the reduced effectiveness of certain antibiotics. This results in life-threatening infections, particularly nosocomial infections where the patient’s immune system is compromised [2]. Novel antimicrobial agents are required to meet the reduced effectiveness of current drug treatments to combat the rise in antibiotic resistance among pathogenic bacteria [2]. However, as outlined in a recent World Health Organisation report, there are very few new classes of antimicrobials in the pipeline against drug-resistant clinical pathogens [3]. Bacteriocins are one potential class that has shown promising activity against antimicrobial drug-resistant infections due to their potency and specificity [3]–[6].

Bacteriocins are antimicrobial peptides produced by bacteria to inhibit competing bacterial strains. There have been a number of bacteriocins to date identified with activity against clinically relevant Gram-positive and Gram-negative bacteria, including bacterial strains which have shown resistance to other drug treatments such as MRSA, *S. pneumoniae*, *Clostridium difficile*, vancomycin-resistant enterococci (VRE) and various mycobacteria [7]–[9]. They are ribosomally synthesised and generally hydrophobic peptides which inhibit related bacterial strains. However, these antimicrobial peptides are subject to many issues such as proteolytic degradation in vivo, low solubility and unfolding and aggregation during storage and formulation [10]. Therefore, modification of these bacteriocins or the development of suitable delivery matrices is necessary to increase their stability. Nisin A (3.4 kDa) is a bacteriocin produced by the Gram-positive lactic acid bacterium (LAB), *Lactococcus lactis* subsp. *lactis* and is classed as a lantibiotic, due to the presence of lanthionine rings within its’ chemical structure. Nisin A is FDA-approved as a food additive (E234) and certified as a GRAS (Generally Regarded as Safe) excipient. It has also shown activity against pathogenic bacterial strains including *S. aureus*, *Escherichia coli*, *S. pneumoniae*, *Enterococcus faecium* and *C. difficile*, indicating its potential for clinical applications [11]–[13]. In addition to its antimicrobial activity, nisin A has also shown immunomodulatory, spermicidal and anticancer activity [14]–[16]. However, the susceptibility of nisin A to proteolytic degradation by intestinal enzymes such as trypsin and α-chymotrypsin, along with poor solubility at certain pH’s have hindered its’ use as an antibiotic treatment [17]–[19]. While there have been some attempts at improving the solubility and stability of nisin A for pharmaceutical applications, none have yet resulted in an FDA-approved dosage form of this antimicrobial [20]–[26].

To improve the stability of nisin A as a food preservative, it has to date been encapsulated in liposomes [27], layered double hydroxides [28] and in numerous polymeric matrices (e.g. chitosan, alginate [29]). While the controlled release of nisin A from these matrices was achieved, evidence for protection from enzymatic degradation is limited. The protective nature of polymeric/hydrogel drug delivery matrices has been previously reported for oral protein delivery [30] [31]. Marschutz & Bernkop-Schnurch examined the protection of insulin against luminally secreted enzymes, i.e. trypsin & chymotrypsin *in vitro* also using polymeric delivery systems. These delivery systems contained polymer-inhibitor conjugates which showed strong inhibitory activity towards degradation of the
hormone by these enzymes [32]. The adsorption/encapsulation approach using these hydrogels is generic and the resulting matrix would be the same for delivery of any peptide or protein, limiting the ability to tailor the delivery matrix for a peptide of a particular size, chemical functionality, activity or instability. This limits the application of these polymeric matrices to proteins or peptides with particular physicochemical properties.

Mesoporous silica (MPS) matrices have previously been used to deliver drug molecules and have also been applied in the areas of bone regeneration, tissue engineering and bio catalysis [33]–[36]. Organic-inorganic hybrid mesoporous matrices, such as periodic mesoporous organosilanes (PMOs), have also been well documented as delivery matrices for small molecules [37] with control over the rate of the release of these small molecules being reported. PMO’s differ from post functionalised or co-condensed mesoporous silicates, where the organic moiety is grafted onto a mesoporous silica matrix or a silica precursor such as tetraethoxy silane is co-condensed with an organically functionalised triethoxysilane group respectively. PMO’s are formed by using an organically bridged silica precursor which ensures that the organic group is homogeneously distributed throughout the matrix and reduces any risk of phase separation. Although it depends on the nature of the organic bridging group, in general, they appear to have a lower haemolytic effect and a higher biocompatibility with cells than pure mesoporous silica matrices [37]. The advantages of using these mesoporous matrices in drug delivery include 1) homogenous distribution of the drug due to the well-ordered porous structure, 2) ease of appropriate organic group functionalisation throughout the matrix and 3) tuning of the pore size, particle size and morphology [37], [38]. Mesoporous matrices have been explored as a potential oral delivery matrix for the enhanced oral bioavailability of poorly soluble drugs, improved dissolution of active pharmaceutical ingredients (API’s) in different pH environments [39][40][41][42]. Thus from our previous work on enzyme immobilisation, we now propose that mesoporous silicates (MPS) and periodic mesoporous silanes (PMO) can be designed as an oral delivery platform to host specific peptides or proteins by tuning their pore size and chemical composition to optimise the interaction between the matrix and the peptide and obtain the optimal loading, release profile and level of protection from enzymatic degradation.

In this study, MPS, SBA-15 [43] and MCM-41[43] (pure silica) and PMO matrices, PMO-PA (amino-functionalised) [44] and MSE (ethylene-functionalised) [45], with pore sizes in the range 2.8-6.8 nm, were studied for their potential to adsorb nisin A and protect it against proteolytic degradation. The adsorption properties of nisin A onto each matrix were explored and the rate of release and activity of nisin A from the porous matrices were analysed. The release of nisin A from the matrices in simulated gastrointestinal media with and without exposure to a degrading protease, and the activity of the nisin A loaded mesoporous particles after prolonged exposure to gastrointestinal media were examined.
2.0 Experimental Section

2.1.0 Materials

Nisin A (95% from *Lactococcus lactis* in sauerkraut) was obtained from Handary, Belgium. Trifluoroacetic acid (TFA, ≥ 99%), acetonitrile (ACN, ≥ 99.9%), sodium taurocholate (NaTc, ≥ 95%), potassium phosphate monobasic (KH_2PO_4, ≥ 99%), L-α-lecithin (≥ 99%, from egg yolk), potassium chloride (KCl, ≥ 99%), hydrochloric acid (HCl, 36.5-38%), acetic acid (99.8-100.5%), sodium citrate dihydrate (≥ 99.0%), citric acid monohydrate (≥ 99.0%), calcium chloride hexahydrate (CaCl_2.6H_2O, 98%), Tetraethoxysilane (TEOS), bis[(trimethoxysilyl)propyl]amine (≥ 90%), 1,3,5-trimethylbenzene (98%), sodium hydroxide (NaOH), ethanol (EtOH), methanol (MeOH), Pluronic P123, Pluronic F127, bis(trimethoxysilyl)ethane (BTMSE), cetyltrimethylammonium bromide (CTAB), bovine serum albumin (≥ 98%), brain heart infusion agar and broth (BHI) were all obtained from Sigma Aldrich Ireland Ltd. Tris base (≥ 99.8%) was purchased from Fisher Scientific Ireland. Sodium acetate was purchased from VWR International Ltd (Ireland). Deionised (DI) water was obtained from an Elga PURELAB System. *S. aureus* (strain 20231) was purchased from the German collection of microorganisms and cell cultures, DSMZ.

2.2.1 – Preparation of MPS matrices

SBA-15 [43], MSE [45], MCM-41 [43] and PMO-PA [44] were synthesised as previously reported and may be found in the supplementary information.

2.2.2 - Characterisation of MPS matrices

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), attenuated total reflectance Fourier transform infra-red (FTIR) and powder x-ray diffraction (P-XRD) analyses is presented in supplementary information.

Solid state nuclear magnetic resonance (SSNMR) analysis was carried out on a Bruker 400 MHz spectrometer at frequencies of 79.49, 100.6 and 400.14 MHz for $^{13}$C, $^{29}$Si and $^1$H respectively using 4 mm ZrO_2 rotors. Optimisation of the magic angles was carried out by spinning a rotor packed with potassium bromide (KBr) at 5 kHz. Samples were spun at 10 kHz. The low field peak of adamantane (38.48 ppm) was used as a reference for $^{13}$C chemical shifts. $^{13}$C cross-polarisation magic angle spinning (CPMAS) spectra were recorded using a contact time of 1-1.5 ms and a recycle delay of 3 s. 1024 scans were collected per sample. Glycine was used to check the signal to noise ratio. The low field peak of 3-((trimethylsilyl)propane-1-sulfonic acid (23.1 ppm) was used as a reference for $^{29}$Si chemical shifts. $^{29}$Si MAS spectra were recorded using a recycle delay of 200 s with 50 kHz line broadening. 300-500 scans were collected per sample. All experiments were conducted with proton decoupling. For SBA-15 and MCM-41, the relative quantities of Qₐ species were determined by
deconvolution of the $^{29}$Si spectra into 3 Gaussian lines using the deconvolution tool in Bruker TopSpin version 3.5pl7.

2.2.3 HPLC analysis of Nisin A
Reverse phase high performance liquid chromatography (RP-HPLC) was used to quantitatively and qualitatively analyse nisin A, using a similar method to Slootweg et al [19]. Analytical RP-HPLC of nisin A was carried out on an Agilent 1260 Infinity system with UV-vis detection operating at 214 nm with a Purospher STAR RP-18 end-capped Hibar (C$_{18}$ 15cm x 4.6 mm, 5 µm) column. The mobile phase consisted of 0.1% TFA (buffer A) and ACN containing 0.1% TFA (buffer B) using a gradient of 75:25 to 55:45 (A/B) over 52 minutes. The chromatographic profile of nisin A showed three peaks (corresponding to the intact nisin A, a modified/oxidised nisin A and a fragment of nisin A). The quantification of nisin A for the loading and release studies was based on the signal from the intact nisin only (Fig. 1, signal B). In order to fractionate the three nisin A components, RP-HPLC analysis was performed on a Kinetix LC-HPLC column (150 x 4.6 mm, 2.6 µm, 100Å) using a gradient of 28:72 to 57:43 (A/B) over 35 minutes. MALDI-TOF MS analysis was carried out on fractions of interest using an Axima TOF2 MALDI TOF mass spectrometer (Shimadzu Biotech, Manchester, UK). 0.5 µL matrix solution (α-cyano-4-hydroxycinnamic acid; 10 mg/mL in buffer A) was deposited onto the target for 5 seconds, then removed. The residual solution was allowed to air-dry and 0.5 µL of the fractions of interest were deposited onto the coated targets. 0.5 µL of matrix solution was added on top of the deposited sample and allowed to air-dry. The sample was analysed in positive-ion reflectron mode.

2.2.4 Nisin A loading onto MPS matrices

2.2.4.1 Initial loading studies
Nisin A solutions (500 µg/mL) were prepared in citrate buffer (100 mM, pH 4.01) or KCl/HCl buffer, (677 mM, pH 2). Mesoporous matrices (10,000 µg/mL; MCM-41/SBA-15/MSE/PMO-PA) were added to the nisin A solution and the nisin A solution without MPS matrices was used as a control. Samples were sonicated until a suspension was formed. While stirring, 15 x 1 mL samples of each mesoporous matrix suspension with nisin A and the nisin A controls were transferred to Eppendorf tubes and placed on a rocker at 37 °C. After 0.5 h, three tubes of each sample and a control were removed from the rocker, centrifuged (5000 rpm, 15 min) and the supernatants analysed for nisin A content by RP-HPLC. This was repeated at 1, 3, 6 and 21 h. The amount of nisin A adsorbed to the mesoporous matrices was then calculated by subtracting the concentration of nisin A in the supernatant from the initial nisin A concentration in solution as determined from the calibration curve. The loading was given in terms of µg of nisin A per mg of mesoporous matrix.

2.2.4.2 Adsorption Isotherms:
Nisin A solutions were prepared at concentrations of 500, 1000, 2000, and 3000 µg/ml in 677 mM, pH 2 HCl/KCl buffer. 5 mg of each silicate (MSE, MCM-41, and SBA-15) was added to 1 ml of nisin A solution and to 1 ml of buffer (as a control). The tubes containing the silicates and controls of free nisin A at each concentration were incubated at 37°C with shaking of 250 rpm for 5 hours, after which they were centrifuged for 15 minutes at 1,500 rpm. The supernatant was analysed using RP-HPLC to determine the concentration of nisin A remaining in solution.

Using the data obtained from the adsorption to the 5 mg of silicate, the adsorption study was scaled up, whereby 100 mg of SBA-15, MSE or MCM-41, were weighed into tubes, and 20 ml of a 2,000 µg/ml nisin A solution was added. The tubes were again shaken at 37°C at 250 rpm for 5 hours. They were subsequently centrifuged, at 5,000 rpm for 15 minutes and the supernatant analysed for nisin A content using RP-HPLC, the pellets were washed with 5 ml of DI water and centrifuged and the pellet wash was also analysed by RP-HPLC.

The adsorption isotherms were fitted to linearised versions of the Langmuir and Freundlich adsorption isotherm theories (equation 1 and equation 2 respectively) and the Langmuir constants for the adsorption coefficient (K) and monolayer surface coverage (C∞) (equation 1) and the Freundlich constants m and a (equation 2) were determined [46], [47].

\[
\frac{C_{eq}}{C_s} = \frac{C_{eq}}{C_\infty} + \frac{1}{KC_\infty} \quad \text{Equation 1}
\]

\[
\log(C_s) = m \log(C_{eq}) + \log a \quad \text{Equation 2}
\]

Where \( C_{eq} \) is the equilibrium concentration of nisin A in solution (µg/ml) and \( C_s \) is the loading of nisin A onto the matrix (mg/g).

### 2.2.5 IC\(_{50}\) of nisin A against S. aureus

*S. aureus* was cultured overnight at 37°C in BHI broth and subsequently diluted to an OD of 0.1. Nisin A was dissolved in a KCl/HCl buffer at pH 2 at a concentration of 500 µg/ml. 200 µl of a bovine serum albumin (BSA) solution at a concentration of 0.1% in PBS was added to the wells of a 96-well microtitre plate and incubated for 30 minutes at 37°C to prevent adherence of the nisin A to the walls of the plate. The BSA was removed and the wells were rinsed with PBS. Varying volumes of the nisin A solution (0.5 mg/ml) were diluted with PBS and added to the *S. aureus* culture (150 µl) to give a final volume of 200 µl and nisin A concentrations of 10, 12, 15 and 20 µg/ml. Controls of BHI broth (200 µl broth), KCl/HCl buffer (44 µl with 150 µl culture), and 150 µl *S. aureus* with 50 µl PBS were added to the plate. The plate was incubated overnight at 37°C in a Biotek ELx808 Ultra microplate reader (Mason Technologies, Dublin), with readings taken every 30 minutes at 590 nm for the 24 hour period. The blank readings were subtracted from the test results, and the median IC\(_{50}\) was
determined based on the concentration that killed 50% of the culture based on the positive control (150 μl S. aureus with 50 μl PBS).

2.2.6 In vitro release study

Modified Fasted State Simulated Gastric Fluid (m-FaSSGF) was prepared by combining 36 ml 1 M sodium chloride, (NaCl), 40 ml 1 mM sodium taurocholate (NaTc), 12.5 ml 1M hydrochloric acid (HCl), 2 ml 5 mM L-α-lecithin. The solution was made up to 500 ml with DI water and the pH was confirmed to be 1.6 [48], [49]. Modified Fasted State Simulated Intestinal Fluid (m-FaSSIF) was prepared with 68.62 mM NaCl, 34.3 mM NaOH, 19.12 mM maleic acid, 0.2 mM lecithin, and 1.618 mM porcine bile extract, the pH was confirmed to be 6.5. There were no enzymes present in the media used for the release studies [48], [49].

Based on the loading onto each matrix, approximately 1-2 mg of nisin A was added to 1 ml of dissolution media by adjusting the amount of loaded matrix added – thus, 5.08 mg of MCM-41-nisin (1,120 μg/ml nisin A as it had a loading of 0.282 mg/g), 33.6 mg SBA-15-nisin (1,820 μg/ml nisin A as it had a loading of 0.0573 mg/g), and 7.14 mg MSE-nisin (1,320 μg/ml nisin A as it had a loading of 0.2277 mg/g), were suspended in 1 ml each of m-FaSSIF and m-FaSSGF with controls comprising of 5.08 mg of MCM-41, 33.6 mg SBA-15, and 7.14 mg MSE, and nisin A solutions of 2,000 μg/ml, 200 μg/ml, and 20 μg/ml, and incubated at 37°C shaking at 275 rpm. Samples were centrifuged at 7,000 rpm for 7 minutes and the supernatant was fully removed at time points of 2, 4, 24, 48 and 72 hours, and replenished with fresh media. The samples were analysed using RP-HPLC to determine the concentration of nisin A present in the supernatant.

The release data was fitted to different release rate equations to theoretically determine the mode of release of nisin A from the matrices. The data was fitted to the zero order (Eq. 3), first order (Eq. 4), Hixon-Crowell (Eq. 5) and the simplified Higuchi (Eq. 6) equations, and the best fit was determined by the regression of the linear plot (R²). The release rate constant (K₀, K, K_HC or K_H) was subsequently determined from the plots produced [50].

\[ Q_t = Q_0 + K_0t \]  
\[ \ln Q_t = \ln Q_0 + Kt/2.303 \]  
\[ 3\sqrt{Q_0} - 3\sqrt{Q_t} = K_{HC}t \]  
\[ Q_t = K_Ht^{1/2} \]

Where \( Q_0 \) indicates the initial concentration of drug (μg/ml), ‘\( Q_t \)’ indicates cumulative drug release (%) at time ‘t’, and time ‘t’ refers to time in hours.

2.2.7 In vitro antimicrobial activity study
A release study was also conducted as described above into m-FaSSIF/m-FaSSGF under sterile conditions, whereby the nisin solution was filter sterilised through a 0.2 µm PES filter, and the MPS matrices were UV irradiated for 30 minutes. 5 mg of unloaded SBA-15, MSE and MCM41, 5 mg nisin A loaded silicates SBA-15-nisin (0.0775 mg/g, thus 359.6 µg/ml nisin A), MSE-nisin (0.0971 mg/g, thus 442.5 µg/ml nisin A), MCM41-nisin (0.0923 mg/g, thus 422.5 µg/ml nisin A) and nisin A (400 µg/ml), were incubated in m-FaSSIF/m-FaSSGF (1 ml) at 37°C shaking at 250 rpm for 4 hours.

The activity of both the supernatant and the pellet from the m-FaSSIF and m-FaSSGF release study was assessed. S. aureus was cultured overnight in brain heart infusion broth (BHI) and diluted to an optical density (OD_{595}) of 0.1. After the 4 hour release, the samples were centrifuged at 13,500 rpm for 7 minutes, and 100 µL supernatant was added to 3 ml of the culture. The pellet was also resuspended in 3 ml of the culture. For the control, 1 ml of the free nisin A solution (400 µg/ml) in FaSSIF was added to 2 ml of culture. The samples were incubated for 4 hours, alongside controls of blank silicates and free nisin A (n=3). After this incubation period, the pellets were centrifuged at 4000 rpm for 15 minutes, and serial dilutions of the supernatant were prepared using PBS and plated on BHI plates (n=4). The plates were incubated at 37°C overnight and the colonies were counted and reported in colony forming units per ml (CFU/ml).

2.2.8 Activity of digested nisin A with S. aureus

In order to determine the activity of nisin A degraded by proteolytic enzyme activity, three 5 ml samples of a 1,000 µg/ml nisin A solution prepared in KCl buffer at pH 2 in the presence and absence of pepsin (1 % w/v) and a solution of 1 % w/v pepsin in KCl pH 2 buffer, were incubated at 37°C for exactly 24 hours. After 24 hours, 5 ml of 10% trifluoroacetic acid (TFA) was added to each sample to halt further enzyme activity. Each sample was subsequently filter sterilised (0.22 µm, PES filter) and 2 ml of each sample was incubated with 3 ml of a S.aureus culture (OD = 0.1) for 4 hours. Serial dilutions were prepared in PBS and plated on BHI plates. Colonies were counted after 24 hours at 37°C and expressed in CFU/ml.

2.2.8.1 Effect of proteolytic enzymes on nisin A adsorbed to MPS matrices

In order to determine the protection offered by the silicates from proteolytic enzymes, a nisin A solution with a concentration of ~ 1,000 µg/ml was prepared in KCl/HCl pH 2 buffer. Mesoporous silicates SBA-15, MSE and MCM-41 loaded with nisin A were added to three tubes such that the nisin A concentration present in the tube corresponded to approximately 1,000 µg/ml. To the loaded silicates, 2.5 ml of a 1% pepsin solution in KCl/HCl was added. Controls of nisin A loaded silicates in pH 2 buffer and free nisin A in both pepsin and pH 2 buffer were run alongside the test samples, each in triplicate. All solutions were shaken at 37°C for 24 hours, after which 2.5 ml of 10% trifluoroacetic acid (TFA) was added to each tube to halt further enzyme activity. The samples were analysed using RP-HPLC to quantify the nisin A present in solution. The pellets were re-suspended in 2.5 ml m-FaSSSGF and shaken at 37°C for 24 hours to de-sorb the remaining nisin A. The samples were
centrifuged at 3900 rpm for 10 minutes, and the supernatant for each sample was analysed by RP-HPLC.

### 2.2.9 Estimation of molecular dimensions

A file corresponding to the structure of pepsin (5PEP) was downloaded from the protein database (http://www.rcsb.org/pdb). The structure for nisin A was not available alone and was only available as its complex with lipid II, a key complex in the active mechanism of antimicrobial peptides, as previously determined by solution NMR (1WCO). Thus the structure was modified and the ligand was removed [51]. The files were viewed using PyMOL (version 2.1.1) and the dimensions were obtained using the Measuring Wizard function in PyMOL.

### 3.0 Results and Discussion

The hypothesis for this work was that ordered porous matrices could protect small peptides from degradation by larger proteases in the gastrointestinal tract and that the order and size of the pores and the chemical composition of the matrices could be optimised to get the optimal loading, protection and release rate of the peptide in vivo. It was hypothesised that smaller pore sizes would protect nisin A from enzymatic degradation and that the chemical functional groups would increase the loading of nisin A onto a particular delivery matrix. It was not clear from in silico studies of nisin A which chemical functionality on the matrix would dominate the adsorption and release processes and thus, experimental data on the loading and the rate of release of nisin A from the mesoporous matrices into modified fasted state simulated gastric fluid (m-FaSSGF) and modified fasted state simulated intestinal fluid (m-FaSSIF) was collected in an attempt to predict the pharmacokinetics of the nisin A formulations in vivo.

### 3.1 Characterisation of Nisin A

HPLC analysis of the as-received nisin A showed the presence of three main components (Fig. 1) with molar masses (Mₜ) of 3370, 3352 and 3152 Da, in order of elution (Fig. 1, inset). The first component to elute (A) was identified as Ser³³-nisin (Mₜ = 3370 Da), where residue 33 is genetically encoded as serine, thus the serine has not been converted to Dha in this variant [52]. The second component (B) corresponded to that of intact nisin A (Mₜ = 3352 Da) and the final component (C) was identified as the nisin A fragment (1-32) (nisin¹⁻³², Mₜ = 3152 Da), in which both the dehydroalanine and the lysine at the C-terminal are cleaved, as reported by Lian et al [53]. All three components appeared to adsorb or release from the matrices studied during this work at the same measurable rate.

The molecular dimensions of nisin A were estimated to be 4.76 nm x 2.32 nm x 0.9 nm and pepsin was estimated to have the dimensions 5.60 nm x 5.54 nm x 4.14 nm. These dimensions for nisin A as measured using PyMOL simply provide a rough estimate of the molecular size and shape of nisin A. Nisin A most likely adopts a different conformation (Fig. 2) in gastrointestinal fluid in the absence of
lipid II but the available structural data gives an estimate of the maximum length of the molecule. The estimated surface potential was determined using the ‘generate vacuum electrostatic function’ in PyMol. The surface potential was estimated to determine the potential for hydrophobic or electrostatic interactions between the enzymes/peptide, and the mesoporous particles. As with the molecular dimensions, the estimated surface potential of nisin A (Fig. 2) is also an estimate deduced from this conformation. The isoelectric point (pI) of nisin A is approximately 8.8 which means that in m-FaSSGF at pH 1.6 and in pH 2 buffer, it would carry a net positive charge [54]. Pepsin has an isoelectric point of 2.2-3.0 [55] and thus would carry a much lower net positive or neutral charge at pH 1.6 or 2. However, the estimated surface potential shows significant areas of positive, negative and neutral charge on both pepsin and nisin. This would indicate that they could interact with a surface through electrostatic or hydrophobic interactions. Nisin A would likely be more attracted to a negatively charged surface than the pepsin. In fact, pepsin appears to have large areas of negative charge from the estimated surface potential which could reduce its adsorption to a negatively charged surface. Pore sizes of approximately 3 nm are likely to accommodate nisin A molecules but prevent the entry of the larger, globular protease molecules such as pepsin, trypsin and chymotrypsin. The selective adsorption of nisin A to porous matrices would ideally protect nisin A against degradation by such proteases.

3.2 Characterisation of mesoporous matrices

Porous delivery matrices with amine, hydroxyl groups and hydrophobic patches were previously synthesised and characterised by our group [44], [56]. Here, these materials were further characterised using solid state NMR (SSNMR) as well as using typical scanning and transmission electron microscopy, X-ray diffraction, nitrogen adsorption and attenuated total reflectance Fourier transform infra-red (FTIR) analysis. The physicochemical properties of the mesoporous matrices are presented in Table 1.

The average pore diameters for SBA-15, PMO-PA and MSE were comparable (6.8, 5.9 and 6.0 nm respectively) with MCM-41 presenting a narrower pore diameter (2.8 nm). The broad pore size distribution for PMO-PA can be attributed to the disordered porous array of this material, as confirmed by TEM and P-XRD analysis (supplementary material) The irregular shape of the PMO-PA particles made it difficult to determine the average particle size by SEM but the other three materials had particle size distributions in the sub-micron range with MCM-41 being the smallest by far with an average particle size of ~170 nm (Table 1).

The chemistry of each matrix was characterised by FTIR and SSNMR spectroscopy. The amine-functionalisation of PMO-PA was confirmed by the presence of the N-H stretch at 3328 cm\(^{-1}\) and the N-H bending at 1641 cm\(^{-1}\) in the FTIR spectrum. The ethylene-functionalisation of MSE was confirmed by the C–H deformation vibrations at 1276 and 1418 cm\(^{-1}\) (data not shown) [57]–[59]. \(^{13}\)C CPMAS SS-NMR analysis of the four mesoporous matrices confirmed the chemical functionalisation
of each material (Fig. 3a). MSE had one peak at a chemical shift (δ) of 5.3 ppm, corresponding to the ethyl carbons. For the PMO-PA material, three peaks were present at 10.2, 20.1 and 50.8 ppm, corresponding to the three environments for the amino propyl bridging carbons. The peak furthest up field most likely corresponds to the carbons bound to silicon, with the peak at 20.1 ppm corresponding to the carbons bound to other carbons and the peak downfield from that at 50.8 ppm corresponding to the carbons bound to the amino groups, due to deshielding of carbon by the more electronegative nitrogen atoms. Similar signals were observed by Hartono et al for their aminopropyltriethoxysilane functionalised silicates [60]. Thus, the organic functional groups, -CH_2-CH_2- and -CH_2-CH_2-CH_2-NH-CH_2-CH_2-CH_2- were successfully incorporated into the silica matrices for MSE and PMO-PA respectively. The ^13^C CPMAS spectrum of SBA-15 and MSE showed small signals (δ ≈ 59 ppm, 15 ppm) indicating the presence of a small amount of residual surfactant (P123), undetected by ATR-FTIR.

^29^Si SS-NMR was also used to confirm functionalisation of MSE and PMO-PA and can distinguish between siloxane species (Si(OSi)_3(OH))_3-n Q_n) and organosiloxane species (T_n). The peaks at δ = -90 to -120 ppm in the ^29^Si-NMR spectra of all four matrices correspond to the Q_n species (Fig. 3b). Three peaks are present in this range corresponding to siloxane bridges (SiO_2, Q_2), silanol ((SiO)_3SiOH, Q_3) and geminal silanol ((SiO)_2Si(OH)_2, Q_2) silicon species [61]. For MSE and PMO-PA, an additional set of peaks at δ = -50 to -70 ppm was present in the ^29^Si-NMR spectra, corresponding to the T_n species present as a result of organic functionalisation periodically dispersed throughout the matrix [62]. The peaks in the Q_n region and the T_n region were integrated to approximate the ratio Q_n to T_n species (Table 2). From this calculation, roughly 94% of T_n type silicon atoms exist in MSE, i.e. those bound to ethane groups, with a small percentage (6%) bound to other silicon atoms via a siloxane bridge (Q_2 species, present as a broad peak) which may arise from Si-C bond cleavage during synthesis. The PMO-PA material contained 30% T_n type silicon species, i.e. those bound to the propyl amine functional groups, and 70% Q_n type silicon species. Both MCM-41 and SBA-15 contained Q_n type silicon species only, as expected. The chemical structures for all of the materials synthesised are shown with the ^13^C and ^29^Si NMR spectra (Fig. 3c-e). While MCM-41 and SBA-15 are both pure mesoporous silicates, the surfactant is removed by calcination for MCM-41 (necessary to ensure complete removal of the CTAB surfactant, a toxic chemical) and by Soxhlet extraction for SBA-15 (to remove the non-toxic Pluronic surfactant P123). From deconvolution of the Q_n species in the spectra into 3 Gaussian lines, the proportion of silanol species (Q_2 and Q_3) was found to be reduced in MCM-41, compared to SBA-15 as a result of calcination (Table 2) giving MCM-41 proportionately more hydrophobic adsorption sites than SBA-15.

From previously published data, the isoelectric points of the mesoporous matrix particles are reported (Table 1)[44], [56], [54], [55], [63]. At pH 2, MCM-41, SBA-15 and MSE are all just below their isoelectric point and thus will have a low positive surface charge. At pH 2, the amine-functionalised
silica, PMO-PA, would be strongly positively charged due to the presence of the amino propyl bridging groups and the higher isoelectric point of this MPS (pI 9.1) [44].

3.3 Adsorption Studies

Based on isoelectric points, it was hypothesised that at pH 2, MCM-41, SBA-15 and MSE would adsorb more nisin A (pI = 8.8) than the more positively charged PMO-PA. Nisin A solutions were not particularly stable at pH 7 and so adsorption studies were not conducted at this pH. MCM-41 had a significantly smaller pore diameter than either SBA-15 or MSE, 2.8 nm versus 6.0 nm and 6.8 nm. Thus, the pores of MCM-41 should be impenetrable by pepsin (5.60 nm x 5.54 nm x 4.14 nm), the proteolytic enzyme used in this study, but nisin A with its smaller dimensions (4.76 nm x 2.32 nm x 0.9 nm) may be able to diffuse through its hexagonal porous network. The pores of MSE, PMO-PA and SBA-15 are large enough to allow pepsin to diffuse to some extent into their porous network, and potentially degrade any nisin A that it can reach.

From the initial adsorption studies, nisin A did not adsorb onto PMO-PA at pH 2-4 but adsorbed significantly onto MCM-41, SBA-15 and MSE. When these latter three matrices were incubated with solutions of increasing nisin A concentration at pH 2, up to 3000 µg/mL, MCM-41 reached a maximum loading of nearly 400 µg/mg while lower loadings were observed onto both MSE and SBA-15. From the adsorption isotherms data (table 3), nisin A is bound more strongly to MCM-41 than MSE and the binding to SBA-15 is the weakest. The isotherms also indicate that at higher concentrations of nisin A, higher loadings onto SBA-15 are observed. However, at concentrations of 2500 µg/ml nisin A or higher, precipitation of the nisin A was observed in the controls. This aggregate formation, potentially even induced earlier than in the control by the heterosurface presence, meant that when the equilibrium concentration of nisin was greater that 2500 µg/ml, an accurate loading could not be determined. The adsorption isotherms (Fig. 4) indicate that more nisin A binds to MCM-41 than to MSE. In addition, the binding of nisin A to SBA-15 appears to be quite weak at lower nisin A concentrations. From the study carried out, maximum loadings of 0.0976 ± 0.0002 mg/g onto SBA-15, 0.258 ± 0.002 mg/g onto MSE, and 0.391 ± 0.005 mg/g onto MCM-41 were obtained.

By fitting the adsorption data to linear models of the Langmuir and Freundlich theories (Table 3) it was found that the MCM-41 and MSE could load significantly higher amounts of nisin A compared to SBA-15 with MCM-41 exhibiting the highest adsorption coefficient (K). From the adsorption isotherm theories, it appeared that nisin A adsorbs predominantly through hydrophobic interactions - with adsorption onto the SBA-15 surface being weaker than onto MCM-41 or MSE, most likely due to the scarcity of hydrophobic moieties on its surface. No nisin A is absorbed onto PMO PA due to the combination of PMO-PA’s hydrophilic and highly positively charged surface.
It appears the smaller pore size and higher proportion of hydrophobic siloxane bridges of the MCM-41 compared to SBA-15 promoted adsorption of the peptide to the matrix. These factors increased the strength of the interaction and the overall loading onto MCM-41. Given that both SBA-15 and MCM-41 have a similar chemical composition, this data implies that the pore size and the relative number of siloxane bridging groups in the matrix have a large effect on the strength of binding and loading capacity of a mesoporous matrix for nisin A. The presence of the ethylene bridging groups homogeneously dispersed in the periodic mesoporous organosilane structure of MSE increased the hydrophobicity of the matrix, and this strengthened the interaction between nisin A and the surface of MSE as well as increasing the loading, compared to SBA-15. Pore volumes showed no correlation between the loading and the available pore volume for each matrix, Table 1. From the loadings of nisin A, up to 400 mg/g on MCM-41 which is the equivalent of ~0.12 µmole/g, the nisin A molecules adsorbed would occupy an approximate volume of \(7 \times 10^{-4}\) cm\(^3\), using the dimensions suggested earlier for each nisin molecule, 4.76 nm x 2.32 nm x 0.9 nm. Thus, there is a high excess of pore volume available on all the mesoporous matrices and pore volume does not influence loading.

It has previously been suggested in the literature that the adsorption of a range of small molecule pharmaceuticals onto SBA-15 was best described by the Freundlich equation [64]. A similar trend was observed here for nisin A onto SBA-15 (Table 3) where it is clear that the Freundlich theory fits the adsorption of nisin A to SBA-15 better, with an \(R^2\) value of 0.937. The percentage adsorption onto SBA-15, MSE and MCM-41 was not affected by scaling up to 20 ml and it was noted that the components of the nisin A being used were adsorbed/desorbed at the same rate i.e. there was no preferential adsorption of any of the individual nisin A components (Fig. 1).

### 3.4 In vitro release and activity studies

The median inhibitory concentration (mIC\(_{50}\)) of nisin A against \(S.\) aureus was determined to be ~ 10 µg/ml over a period of 24 hours at 37°C (Fig. 6). The mIC\(_{50}\) of nisin A against \(S.\) aureus was determined in order to ensure that an effective inhibitory concentration of nisin A was adsorbed onto the mesoporous materials for studying their in vitro bioactivity. The rate of release of nisin A from the mesoporous matrices into both modified fasted state simulated gastric fluid (m-FaSSGF) and modified fasted state simulated intestinal fluid (m-FaSSIF) was carried out in an attempt to predict the pharmacokinetics of the nisin A formulations in vivo. Since the rate of dissolution of mesoporous silica matrices in aqueous solution is extremely slow, the release of nisin A from these matrices is primarily due to dissociation from the surface of the matrix or diffusion out of the pores [65].

After 5 h in m-FaSSGF, a burst release of ~10 % and ~2 % nisin A was observed from MSE and SBA-15 respectively, with release from MSE levelling off at ~14 % (Fig. 5). Nisin A continued to release from SBA-15 gradually up to 72 hours. Nisin A release from MCM-41 was faster, with a release of ~21 % after 5 hours and over 28 % by 72 hours. Over 72 % of the loaded nisin A remained
adsorbed to all three matrices despite immersion for 72 hours in both m-FaSSGF and m-FaSSIF. The burst release of nisin A observed from the matrices into m-FaSSGF could be due to nisin A at the surface dissociating from these matrices, followed by the slow diffusion from inside the porous matrix as seen previously for the release of drugs from unfunctionalised silica matrices [34], [66]. The higher cumulative release from MCM-41 in this media compared to SBA-15 may be due to the smaller particle size of MCM-41, with its shorter pores resulting in smaller diffusion distances, as well as the increased external surface area of the MCM-41 particles which could possess a greater concentration of surface adsorbed nisin A. Additionally, in this release study, the loading per mg of MCM-41 and MSE were much higher than the SBA-15 loading which can influence the dissolution profile. Further studies are being conducted to probe the influence of loading on the dissolution profile of nisin A from MSE. Conversely, MCM-41 showed the highest release and adsorption indicating that the nisin A may be interacting with the MCM-41 through hydrophobic interactions, thus showing increased dissociation. This is similar to the release of LL-37, another cationic AMP, at pH 7.4 from silanol rich silica matrices with pore sizes of 3.1-3.5 nm observed by Braun et al [67].

The in vitro release data into m-FaSSGF from all three matrices was found to best fit Higuchi’s release model (Supplementary Material). This is similar to several previously reported drug release kinetics studies from mesoporous matrices [68]. The Higuchi model describes release kinetics which slow as the diffusion path or the distance that the drug molecules have to travel increases [50]. This finding supports the hypothesis that the burst release observed from the data sets indicates the presence of surface adsorbed nisin A which dissociated from the surface upon suspension in the m-FaSSGF, followed by a slower diffusion of nisin A from the pores of the mesoporous matrices. No release of nisin A was detected by RP-HPLC analysis from SBA-15, MSE or MCM-41 into m-FaSSIF over this time period, due to the poor solubility of nisin A at the pH of the m-FaSSIF (pH 6.5).

The activity of the supernatants and pellets against S. aureus was studied. During the activity assays, all of the nisin A loaded matrices and their supernatants in m-FaSSGF completely inhibited the growth of S. aureus. The activity of the pellets from the m-FaSSIF release showed that the silicate pellets showed an 8 log_{10} reduction, 8 log_{10} reductions and 9 log_{10} reductions (SBA-15-nisin, MSE-nisin, and MCM-41-nisin respectively (Fig. 7)). The m-FaSSIF supernatant showed no killing of the bacterial culture after the 4 hour time period, with no decrease in colony forming units per ml based on the positive control (n=4). No inhibition was observed from the m-FaSSIF or the blank silicate (supernatant or pellet) controls (n=4). The activity exhibited by the MSE-nisin and the SBA-15-nisin pellet was comparable, with the MCM-41-nisin pellet showing slightly higher inhibitory activity. The activity shown by the pellets indicates that the nisin A loaded mesoporous particles themselves were active when dispersed near the bacteria, presumably due to the diffusion of the bacterial cells toward the active particles, and the slow release of the bacteriocin from these matrices. This activity from the
pellets could be advantageous when administered near the site of infection and provide protection from any degrading enzymes in the intestinal tract.

3.5 Protection of Nisin A Offered by Mesoporous Matrices

The protection offered by the mesoporous matrices was tested with pepsin. Quantification post incubation with pepsin showed that ~21 % of intact nisin A remained in solution after the 24 hour period, in comparison with the control of nisin A at pH 2 for 24 hours in the absence of pepsin.

The protection offered by SBA-15, MSE and MCM-41 was then determined by suspending nisin A loaded MPS matrices in a pepsin solution. After 24 hour incubation, the concentration of intact nisin A in the supernatant decreased by 54.4 %, 43.1 % and 41.9% from SBA-15-nisin, MSE-nisin and MCM-41-nisin, respectively compared to release into the KCl/HCl pH 2 buffer without pepsin (Table 5). After re-suspension in m-FaSSGF, the MSE-nisin showed a higher release of intact adsorbed nisin A, in comparison to the nisin release from the MCM-41. It is possible that either (i) pepsin had degraded more of the nisin A adsorbed in the MCM-41 and SBA-15 matrices or (ii) the pepsin molecules adsorbed to the surface of the MCM-41 and SBA-15, blocking diffusion of nisin A from the pores. In any case, no intact nisin A was diffusing into the m-FaSSGF media from SBA-15 which would reduce its antimicrobial potential.

The pore size is likely a defining factor in terms of protection based on the molecular dimensions of the pepsin as previously discussed. However as indicated by the results, surface functionality is also an influential aspect. The MSE has slightly smaller pore size than the SBA-15 (6.0 nm) and contains hydrophobic residues (-CH₂-CH₂-) which may contribute to the increased protection in comparison to the SBA-15. The interaction of pepsin with the matrices needs to be further probed to establish if a high affinity of the proteases for the matrix reduces or enhances the antimicrobial activity of the nisin A loaded matrix. Thus, with regard to protecting the peptide against enzymatic degradation, reducing the pore size and increasing the hydrophobicity of the matrix improved protection of nisin A against pepsin.

4.0 Conclusions

The adsorption properties of nisin A onto four different mesoporous matrices were explored and the rate of release of nisin A from the porous matrices was analysed. Chemical functionality and pore size had a large influence on loading, release rate, activity and protection from enzymatic degradation. The loaded matrices and the released nisin A were both found to exhibit antimicrobial activity. Specifically, the bacteriocin, nisin A, can be successfully adsorbed onto pure silica and ethylene-functionalised but not amine-functionalised silica matrices. Unfunctionalised calcined silica with a small pore size (< 3nm) exhibited the strongest binding, the highest loading, and offered the peptide a high degree of protection from enzymatic degradation in bio-relevant media. Conversely, a more
hydrophobic periodic mesoporous organosilane exhibited a lower loading but offered a similar degree of protection with a higher release of intact nisin A after exposure to pepsin. Thus a mesoporous matrix can be designed, in terms of its pore dimensions and its chemical functionality, to control the loading, the rate of release and the stability of a particular bacteriocin into bio-relevant media. This antimicrobial formulation strategy could be used for a wide range of bacteriocins. It is predicted that depending on the surface properties and size of the peptide, a more/less hydrophilic or larger/smaller pore matrix could be formulated to optimise the loading, release and protection offered by the porous matrix. Thus mesoporous silica-based matrices offer potential as a novel formulation approach for bacteriocins and could potentially enable their development into novel antimicrobial medicines.

**Funding**

This work was financed by Science Foundation Ireland under the Bioavailing of Antimicrobial Resources (BAR) project (13/CDA/2122).

**Acknowledgements**

Pauric Bannigan, Amrit Kumar, and Sarah Foley/Ger Bree, from the Bernal Institute, University of Limerick, are gratefully acknowledged for their help in running the PXRD, nitrogen adsorption and calcination, respectively.

**Declarations of Interest**

None

**5.0 References**


2016.


Figure 1. RP-HPLC chromatogram of Nisin A (1 mg/mL) with MALDI-TOF weight determinations of A, B & C of 3370, 3352, and 3154 Da, respectively (inset).
Figure 2. Structures and estimated surface potentials at pH 7 (extracted from the Protein DataBank (PDB) and viewed using PyMOL) of (a) pepsin (5PEP), and (b) nisin A (1WCO, modified to remove the peptidoglycan ligand, lipid II).
Figure 3. From top to bottom: (a) $^{13}$C CPMAS spectra for PMO-PA, MSE, MCM-41 and SBA-15, (b) $^{29}$Si MAS spectra of PMO-PA, MSE, MCM-41 and SBA-15 and chemical structure of (c) PMO-PA, (d) MSE and (e) MCM-41 and SBA-15
Figure 4. Adsorption isotherm of nisin A onto SBA-15, MSE and MCM-41 at pH 2 (677 mM, pH 2 KCl/HCl), at 37°C. (Y error bars included)
Figure 5. Percentage release of nisin from SBA-15-nisin (1.82 mg nisin A available), MSE-nisin (1.32 mg nisin A available), and MCM-41-nisin (1.12 mg nisin A available) into 1 ml m-fasted state simulated gastric fluid (m-FaSSGF) over time at 37 °C.
Figure 6. Antimicrobial activity of nisin A against *S.aureus* at concentrations of 10, 12 & 15 µg/ml nisin after 24 hours at 37°C.
Figure 7. The activity of nisin-loaded matrices against *S. aureus*, free nisin (174 μg), supernatants and pellets from the m-FaSSIF release study. The silicates; SBA-15, MSE and MCM-41 are nisin loaded particles, whereby SN is the supernatant and P is the pellet.
Table 1. Summary of the properties of the as-synthesised mesoporous silica matrices.

*Calculated from BJH desorption curve of adsorption isotherm

Table 2. Relative abundance of Qₙ and Tₙ Si species in MPS, approximated by integration on single pulse ²⁹Si MAS spectra and deconvolution of the Qₙ species into 3 Gaussian lines.

Table 3 Parameters obtained by fitting the nisin adsorption to SBA-15, MSE and MCM-41 data to Langmuir and Freundlich linearized isotherms. The measure of fit to the model is indicated by the R² value.
<table>
<thead>
<tr>
<th>Mesoporous Matrix</th>
<th>Model of Best Fit</th>
<th>Equation</th>
<th>$R^2$</th>
<th>Higuchi Constant ($K_H$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBA-15-nisin</td>
<td>Higuchi Kinetics</td>
<td>$Q_t = K_H t^{1/2}$</td>
<td>0.97</td>
<td>1.11</td>
</tr>
<tr>
<td>MSE-nisin</td>
<td>Higuchi Kinetics</td>
<td></td>
<td>0.74</td>
<td>0.85</td>
</tr>
<tr>
<td>MCM-41-nisin</td>
<td></td>
<td></td>
<td>0.90</td>
<td>1.59</td>
</tr>
</tbody>
</table>

Table 4 Calculated Higuchi constants for the in vitro release kinetics from SBA-15-nisin, MSE-nisin and MCM-41-nisin.

Table 5 The percentage protection of nisin A by mesoporous matrices based on the difference between detection of nisin A in KCl/HCl with and without pepsin (1% w/v).

<table>
<thead>
<tr>
<th></th>
<th>SBA-15-Nisin</th>
<th>MSE-Nisin</th>
<th>MCM-41-Nisin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass of nisin A loaded matrix added</td>
<td>$49.9 \pm 1.4$ mg</td>
<td>$37.4 \pm 0.3$ mg</td>
<td>$36.9 \pm 0.5$ mg</td>
</tr>
<tr>
<td>Total nisin A present</td>
<td>$4,590 \pm 130$ µg</td>
<td>$4,281 \pm 31$ µg</td>
<td>$4,341 \pm 48$ µg</td>
</tr>
<tr>
<td>Released in 1.0% w/v pepsin in KCl/HCl, 24 hours, at 37°C</td>
<td>$570.5 \pm 46.3$ µg</td>
<td>$355.6 \pm 17.1$ µg</td>
<td>$472 \pm 127.6$ µg</td>
</tr>
<tr>
<td>Typical release into KCl/HCl, 24 hours, at 37°C</td>
<td>$1,252 \pm 220$ µg</td>
<td>$625.5 \pm 63.4$ µg</td>
<td>$811.7 \pm 80$ µg</td>
</tr>
<tr>
<td>The percentage decrease in intact nisin in pH 2 KCl/HCl after 24 hours exposure to pepsin.</td>
<td>$54.4 %$</td>
<td>$43.1 %$</td>
<td>$41.9 %$</td>
</tr>
<tr>
<td>Released in m- FaSSGF after exposure to 1% pepsin at the 48 hour timepoint, at 37°C</td>
<td>Not detectable</td>
<td>$641 \pm 12$ µg</td>
<td>$232 \pm 22$ µg</td>
</tr>
</tbody>
</table>
Graphical abstract

Protection of nisin from protease degradation by mesoporous matrix

In Vitro Release & Bioactivity