



# Bioactivation of PET woven fabrics using alginate biopolymer and the bacteriocin nisin

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## Abstract

Bioactive antibacterial PET (polyethylene terephthalate) woven fabrics were produced by fixing a thin alginate coating with entrapped nisin at the PET fiber surface. These textiles may find applications in the field of hygiene or food packaging. In the first part of the study, thin solid alginate films (without nisin) capable of forming hydrogels in aqueous medium were deposited at the PET fiber surface and the properties of the resulting textiles characterized. Methods using padding with CaCl<sub>2</sub> solution before or after sodium alginate deposition, with or without a prior PET fiber surface activation with air atmospheric plasma, were used. The following properties of the functionalized PET textile were tested: the durability and cohesion of the alginate film to the fiber surface, in water and in dry conditions under friction; the surface properties of the functionalized textile; and the water and moisture absorption capacity of the alginate films. These properties depend on the film adhesion to PET fibers and on the degree of alginate crosslinking. Entrapped bioactive nisin in the alginate film imparted antibacterial properties to the woven polyester fabric against *Staphylococcus aureus*. However, differences in inhibition zone were due to the method of alginate film formation, which had an impact on the rate of release of nisin.

## Keywords

PET textile, alginate film, calcium chloride, adhesion, plasma treatment, nisin, antibacterial properties

Having a biofunctionalized porous textile structure is essential for diverse applications such as apparel, hygiene (for protection ex: gowns, respiratory masks), or food packaging. The use of alginate films immobilized on a textile structure would be an interesting way for producing a biofunctionalized porous structure since alginate films are able to entrap and release bioactive substances in particular conditions.<sup>1–3</sup>

Alginate hydrogels have already been used for immobilization of proteins (such as growth factors),<sup>4</sup> and food grade alginates have been extensively used for edible coating. Moreover alginate in the form of fibers is used for wound dressing.<sup>5</sup> Alginate is a natural polysaccharide containing various proportions of manuronate and guluronate monomers which are linked by glycosidic bonds (Figure 1(a)). Guluronate monomers in one alginate molecule can be linked to a similar region in another alginate molecule by means of

calcium ions to form a gel.<sup>6</sup> Drugs or antimicrobials can thus be immobilized in alginate gels and released under certain conditions.<sup>1–3</sup>

Nisin (Figure 1(b)) is a bioactive antibacterial agent already used for edible food coatings such as alginate films.<sup>7</sup> Nisin is an antimicrobial peptide produced by strains of *Lactococcus lactis* subsp. *Lactis*, and used as a biopreservative and a potential agent in

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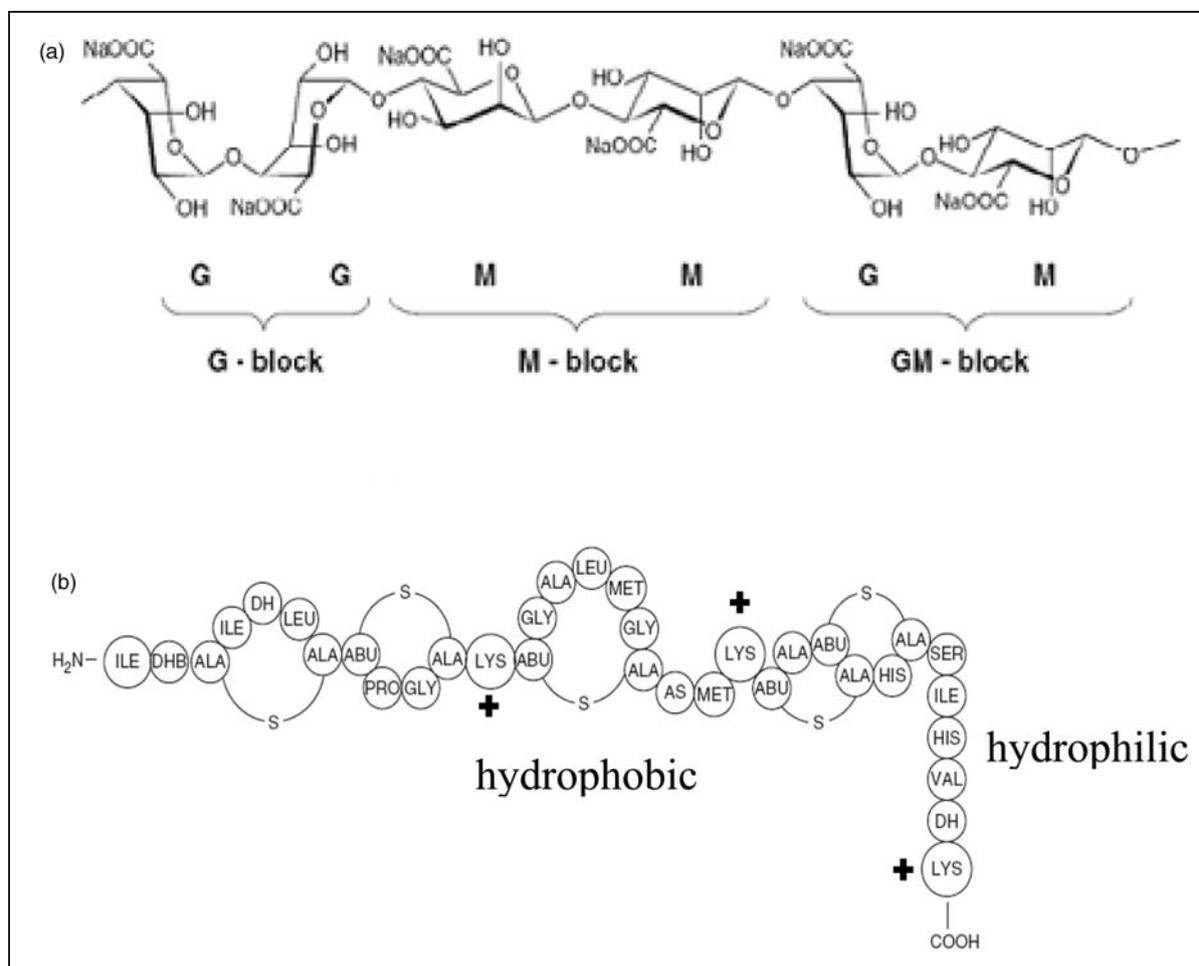
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pharmaceutical, veterinary, and health care products. Nisin has a cationic character depending on the pH.<sup>8</sup> It is a 34 amino acids peptide with a molecular mass of 3500 Da. Nisin can inhibit the growth of Gram-positive bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, and *Bacillus cereus*.<sup>8,9</sup> In addition, nisin was affirmed generally to be recognized as safe (GRAS) by FDA in 1988, and it is now used as a biopreservative in many countries around the world.<sup>10,11</sup> Since nisin can inhibit the growth of pathogenic bacteria, textile functionalization using nisin may provide a good tool for preventing microbial attachment, and cross contamination. Antibacterial property of the nisin is given by the diffusion ability of the bacteriocin, and it kills susceptible bacteria through a multi-step process that destabilizes the phospholipid bilayer of the bacterial cell and creates transient pores as described by Lins et al.<sup>12</sup>

In the present study, the possibility of functionalizing a woven PET fabric using alginate and entrapped bioactive nisin is investigated. The biofunctionalized

porous textile can be used for applications such as apparel, food packaging or hygiene application.

PET fiber surface activation using air-atmospheric plasma treatment has been used together with textile finishing techniques (padding) to deposit an alginate film without considerably blocking textile pores. Porosity and pore size determine the air permeability which is an important attribute in applications such as apparel or respiratory masks for physiological comfort, or in certain types of food packaging. In the first part of this study, different methods for forming thin alginate films on the PET fibers were investigated. The physical properties (film durability to aqueous conditions and to mechanical strain such as friction) and physico-chemical properties of the functionalized fabric (wettability, moisture and water absorption) were studied to understand the alginate layer adhesion and structure at the fiber surface. In the second part, the antibacterial properties against *S. aureus* of textiles functionalized by entrapping bioactive nisin in the alginate film were investigated and related to the alginate film properties.



**Figure 1.** Chemical structures of sodium alginate (a) and nisin (b).

## Materials and methods

### Materials

**Polyethylene terephthalate fabric structure.** A 100% polyethylene terephthalate (PET) woven fabric of density 120 g/m<sup>2</sup>, with 24 yarns/cm in warp direction and 20 yarns/cm in weft direction, with a thickness of 0.56 mm and a porosity of 63.5% was used for the study. The PET woven fabric was cleaned to be free from surface impurities and spinning oil.

**Plasma treatment of woven PET samples.** Plasma treatment of the PET fabric was carried out using an air-atmospheric plasma machine called 'Coating Star' manufactured by Ahlbrandt System (Germany). The following machine parameters were kept constant: electrical power of 1 KW, frequency of 26 KHz, speed 2 m/min, electrode length of 0.5 m and inter-electrode distance of 1.5 mm. After plasma treatment at 60 KJ/m<sup>2</sup>, the plasma treated sample was kept in aluminum foil away from light to avoid ageing effects.

### Chemicals

**Alginate.** Commercial grade Alginate LAMITEX L10 (low molecular weight), which is also used for printing of textiles, was purchased from BFC.

**Nisin.** Nisaplin was purchased from Sigma-Aldrich (France) as a commercial preparation containing 10<sup>6</sup> IU/g of nisin. A 10<sup>4</sup> IU/ml stock solution of nisin was prepared by dissolving 100 mg of the nisaplin preparation in 10 ml of 100 mmol/l HCl and stored at 4°C.

### Methods

**Alginate thin film formation.** Thin alginate films were formed using three or four of the different steps described below:

Step 1. Air-atmospheric plasma treatment.

Step 2. Padding of the fabric in an aqueous solution of sodium alginate (6 g/l).

Step 3. Padding of the fabric in aqueous CaCl<sub>2</sub> solution (2.5 g/l).

Step 4. Drying in air at room temperature and pressure (RTP).

Padding was used to deposit the sodium alginate or the calcium chloride: the fabric was impregnated with the aqueous solution followed by squeezing out by passage through rotating cylinders at a pressure of 4 bar. Indeed, without squeezing of the fabric, a thick gel coating is formed at the fabric surface which on drying forms a non-adhering solid film at the fabric surface and which is easily crashed into dust.

Two different methods were used for calcium alginate film formation with or without step 1 (plasma pre-activation):

Method 1 (M1), step 3 → step 2 → step 4

Method 2 (M2), step 2 → step 3 → step 4

From the wet pick up (see Table 1), the total amount of alginate on the fabric is very small: nearly 0.4 % w/w for either methods M1 or M2. Moreover, after functionalization with alginate, there was only a small reduction in air permeability (measured according to the standard test ISO 9237), as shown in Table 2.

The PET surface pre-activation with air-atmospheric plasma increased the wet pick-up rate (see Table 1) and the amount of alginate deposited increases to around 0.7%. Accordingly, there is a higher reduction in air permeability but the fabric is still very porous; the more hydrophilic nature of the plasma treated PET fiber surface would explain the higher wet pick-up of alginate solution.<sup>13,14</sup>

### Physico-chemical characterization of PET fabrics functionalized with alginate films

**Alginate thin film durability.** Wet durability tests of the PET fabric functionalized with thin alginate film were carried out after dipping the fabric sample in a water bath for 30 min, at different temperatures (20°C, 40°C,

**Table 1.** Quantification and properties of alginate films deposited at the PET textile surface and their resulting properties

Method for calcium alginate film formation	Wet pick-up rate (%)	Quantity of alginate (%) w/w of PET fabric	Moisture absorption (%) in w/w of alginate film (at RH of 60%)	Water absorption (%) in w/w of alginate film	Quantity of Ca <sup>2+</sup> ions (mmol/l) during rinsing in water for 30 min
M1	74	0.44%	164	1803	0.355
Plasma M1	120	0.72%	130	620	0.177
M2	68	0.41%	41	650	0.355
Plasma M2	112	0.67%	132	630	0.355

**Table 2.** Water contact angle and capillarity (%) of PET fabric without and with alginate films

		Air permeability (l/m <sup>2</sup> /s)	Water contact angle (°)	Capillarity (%)
PET (without alginate films)	PET	3000 ± 50	68 ± 5	79 ± 40
	plasma treated PET	3000 ± 50	45 ± 10	156 ± 60
PET with calcium alginate films	M1	2740 ± 50	59 ± 5	126 ± 50
	plasma M1	2100 ± 40	36 ± 2	177 ± 70
	M2	2625 ± 40	66 ± 5	96 ± 75
	plasma M2	2210 ± 50	55 ± 2	145 ± 70

and 60°C). A dry rub durability test was carried out using a Crockmeter and the standard test ISO 105-X12.

To measure the alginate film durability, 2 g/l of a cationic dye (Blue Sandocryl B-RL) were incorporated into the aqueous solution of sodium alginate before application to textiles, and then the color loss was quantified after each durability test. The color degradation was evaluated using the Datacolor Spectraflash® SF 600 using a grading scale of 5 units (Grade 5: means no color loss). As strong ionic bonds exist between the cationic dye and the negatively charged alginate polyelectrolyte, color loss is supposed to be due to loss of the alginate film, i.e. loss of film adhesion, rather than loss of dye. Complementary experiment shows that, after immersion in water of a solid gel capsule of dyed calcium alginate, the dye does not migrate from the capsule into water.

**Moisture and water absorption properties.** The test consists of quantifying the moisture absorption of the alginate film (%) by measuring the weight gain after having subjected the samples to drying in a desiccator for 24 h and then in an air-conditioning chamber (CLIMATS) with a relative humidity of 60% for a period of 24 h. The difference in weight between the PET fabrics with and without alginate film gives the moisture absorption by the alginate film. Four trials were carried out for each sample.

For water absorption capacity measurements, four vertically hanging rectangular-shaped PET fabrics were immersed simultaneously at a constant speed in a beaker containing distilled water, for 3 min. The samples were then removed from water at a constant speed and then subjected to squeezing using a wringer. The increase in weight due to water absorption by alginate film was calculated by using water absorption values of the PET fabrics without and with an alginate film.

**Wettability of the functionalized fabrics.** Water contact angle and capillary weight measurements were carried out on a tensiometer, 3S Balance from GBX

Instruments (France), as described in our previous work.<sup>13,14</sup>

Prior to wettability tests, all samples were rinsed in water to remove all unbound species. A rectangular fabric sample 3 cm × 5 cm was immersed in 50 ml of distilled water and the conductivity of the rinsing water measured in µs/cm.

After removal of unfixed species, the water wettability of three different samples was tested and their water contact angle and capillary weight (at 6 min) measured.

**Entrapment of nisin in alginate films immobilized at the PET fabric surface.** The two methods M1 and M2 were used to functionalize PET with alginate containing nisin. First 10 g/l of Nisaplin solution was mixed with 6 g/l sodium alginate solution at pH3. This pH was the lowest pH ensuring stability of nisin without allowing acidic gelification of alginate solution. Then polyester fabrics were functionalized using methods M1 and M2 with the mixture of (alginate + nisin) solution, instead of alginate alone.

**Antibacterial characterization of nisin treated PET using a diffusion method.** An overnight culture of *Staphylococcus aureus* was diluted 200 times in Müeller Hinton medium. 2 ml of this dilution was transferred to a Chapman mannitol salt agar Petri dish, spread evenly over the plate and the excess removed. The Chapman mannitol salt agar medium contains phenol red which turns yellow upon fermentation of D-mannitol by the bacterium. The textile sample was placed in the center of the plate and all plates were incubated at 30°C in a humid chamber overnight. The next day any zone of inhibition around the samples was measured. The antibacterial activity was indicated by an inhibition zone around the functionalized PET fabric discs (diameter of 3 cm) and by the color of the fabric which remains red if there is no bacterial growth under the PET fabric.

**Release of nisin in water.** Experiments carried out in our laboratory showed that the presence of released

alginate or calcium from the polyester fabric into the rinsing water, did not change the water surface tension. Thus the quantity of nisin released into the water was followed by tensiometry. The water surface tension varies linearly with the logarithm of nisin concentration (see Figure 7(b)).

## Results and discussions

### Part I: Alginate thin film characterization

**Alginate thin film durability.** Film durability tests were carried out on PET functionalized with alginate film containing the cationic dye.

Higher wet durability in water as well as higher dry durability to rubbing are obtained with method M1 compared to method M2 (see Figure 2). Thus, the wet durability index value at 20°C is only around 1 for method M2, and around 2.5 for method M1. For method M1, the use of plasma increases further the wet durability index to nearly 4.

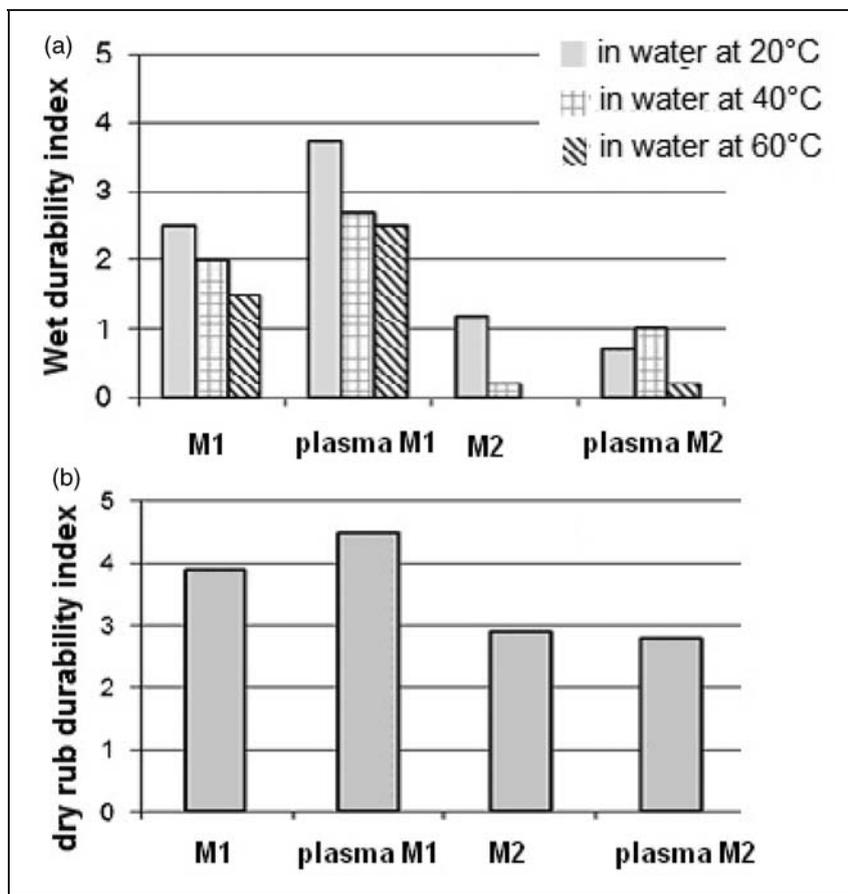
These results show that the alginate layer in method M1 adheres better to the PET fiber surface than method M2.

Plasma treatment seems to improve the water and dry rub durability of the alginate film formed by method M1 only. However, this is not the case with the alginate film formed using method M2.

**Characterization of moisture and water absorption properties.** Table 1 presents the average values for moisture and water absorption capacity of the functionalized textiles. Results are presented in percent weight per weight (w/w) of alginate film deposited on the textiles.

Whatever the treatment, the water and moisture absorption of the alginate film are high. These data confirm the hydrogel forming characteristics of calcium alginate which are qualified as superabsorbent gels.

Without plasma treatment, the network formed by successive application of CaCl<sub>2</sub> and alginate on untreated PET (M1), gives the best results as far as



**Figure 2.** Film durabilities. (a) Wet durability index in water at different temperatures. (b) Dry rub durability index of PET fabrics functionalized with alginate films.

moisture absorption is concerned, 130% to 160%, and has also the highest water uptake (1800%). In this case, the degree of crosslinking should be the least compared to other alginate films.

On the contrary, the alginate film network formed when alginate and  $\text{CaCl}_2$  are successively applied onto untreated PET (M2), has the least moisture absorption value: 41%.

**Durability in water of the alginate film.** Before performing water contact angle and capillarity measurements, all unfixed species ‘alginate and calcium ions’ were removed by rinsing the functionalized textiles in distilled water. The quantity of released species was assessed by conductivity measurements of the rinsing water (see Figure 3).

The highest rate of release was achieved during the first 30 min rinse. After the fourth rinsing, the very small value of conductivity of rinsing water confirmed that there was no more unfixed species (alginate or  $\text{Ca}^{2+}$  ions) released in water. The quantity of  $\text{Ca}^{2+}$  ions in the first rinsing water was also quantified using water hardness test (Table 1).

With method M1, for both PET and plasma treated PET, low conductivity of rinsing water indicated that there was small amount of unfixed species released. On the contrary, with method M2 high conductivity of the rinsing water measured indicated that higher quantity of unfixed species were released. With plasma, the higher wet pick-up rate of the alginate could explain the higher quantity of released alginate.

After the four rinsing cycles, wicking test was performed on functionalized fabrics.

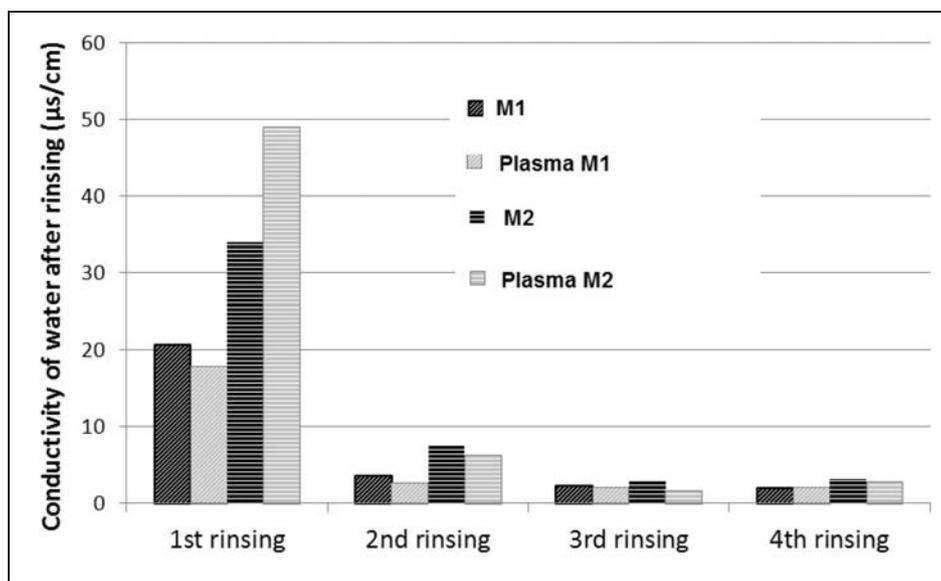
**Water contact angle and water uptake by capillarity of alginate coated fabrics.** PET fabric functionalization with calcium alginate films using method M1 leads to an increase in hydrophilicity of PET fabric compared to method M2 (Table 2). After M1 treatment, the water contact angle decreases from  $68^\circ$  to  $59^\circ$  and the percentage capillarity increases from 79% to 126%.

Plasma treatment alone increases the hydrophilicity of PET fabric ( $\theta = 45^\circ$ , capillarity = 156%) when compared to untreated PET. After application of the alginate film using method M1, there is further increase in hydrophilicity of the polyester fabric ( $\theta = 36^\circ$ , capillarity = 177%).

For PET fabric functionalized with calcium alginate using method M2, there is no increase in hydrophilicity. Without plasma treatment, the calcium alginate film is most probably completely removed after the four successive 30 min rinsing, since the water contact angle of the alginate coated PET is almost similar to that of the uncoated PET fabric surface.

**Discussion (part 1).** The results described above can be explained by the role played by  $\text{Ca}^{2+}$  ions, on the one hand, at the PET fiber and alginate film interface and on the other hand, at the other extreme surface of the alginate film in contact with air (see Figure 4).

When calcium salt is used before alginate deposition (M1), the calcium ions would bind to the carboxylic



**Figure 3.** Release of unfixed species in distilled water by successive 30 min dipping in water, quantified by conductivity measurements.

groups of both the alginate polymer and PET surface. Indeed PET bears end-terminal -COO- (carboxylic) groups, the number of which increases when PET is plasma treated.<sup>13</sup> Thus, when the calcium salt is applied before sodium alginate (M1), these divalent ions would link the alginate film to the PET surface and there would be thus a good film adhesion onto PET fiber surface. These conditions are also the most favorable to prevent release of the film in water and to obtain good wet and dry rub durability results.

In addition, as part of the calcium ions are immobilized at the interface between the PET and the alginate film, there would be more free hydrophilic -COO- groups available within the alginate film. These groups are associated with Na<sup>+</sup> counter ions. This would increase water and moisture absorption of the film, as well as the wettability of fabrics. This film has the highest rate of water absorption (1800%) owing to the greater amount of these free -COO- available.

For the plasma treated PET which is more hydrophilic, there is a higher wet pick-up rate of calcium salt than untreated PET, so in addition of participating in the bonding of alginate film to PET surface, the excess of Ca<sup>2+</sup> ions would migrate through the alginate film and form links with the -COO- groups of the alginate. This would also explain the lower release of Ca<sup>2+</sup> ions during rinsing for plasma M1 (Table 1). So, compared to the method M1 without plasma, the alginate film crosslinking increases and the number of free -COO- in alginate film decreases and this would explain why results in terms of water and moisture absorption are lower.

Without calcium ions at the fiber/alginate film interface (M2), the alginate film is completely released in water after prolonged contact with water and this is even true in the case of a prior plasma surface activation of the PET fiber surface. The majority of the Ca<sup>2+</sup> divalent ions would bind to -COO- free groups of the

alginate, forming a film of calcium alginate with high degree of crosslinking, which would explain the low moisture absorption of the alginate film (41%). The degree of crosslinking is so high that it hinders the migration of excess Ca<sup>2+</sup> ions through the alginate film down to the PET/alginate interface. So the alginate film does not adhere to the surface of PET fiber. Even, a plasma treatment of the PET does not allow an improved adhesion of the alginate.

The four methods of film formation yield alginate films with different degree of crosslinking and different degree of adhesion to fiber surface. It would be worthwhile studying the release of nisin entrapped in the different alginate films formed by the four different methods.

## Part 2: Behavior of nisin entrapped in alginate films immobilized at the PET fabric surface

**Antibacterial characterization of the nisin treated PET.** Whatever the functionalization method used, all PET fabrics with immobilized nisin using alginate films exhibited antibacterial activity against *Staphylococcus aureus* (see Figure 6). In all cases a red coloration was observed around and underneath the PET fabric, which indicated that there was no fermentation of the mannitol by *S. aureus* and by the way no bacterial growth on the PET fabric. Indeed, no red coloration and no inhibition zone were observed for a fabric padded with an aqueous solution at pH 3, which means that antibacterial activity is due to nisin activity alone. Larger inhibition zone was depicted in method 2 (M2) where the CaCl<sub>2</sub> was applied on the final stage (see Figures 5 and 6).

**Release of nisin in water.** Figure 7(a) shows the decrease with time of water surface tension due to release of nisin in water. Each plot represents the mean value for release tests carried out on three different samples of functionalized PET fabrics. The water surface tension is related to nisin concentration in water as shown in the calibration curve in Figure 7(b).

Results show that the release of nisin in terms of quantity and duration time depends on the nisin immobilizing method (M1 or M2).

Thus, with method M1, the quantity of nisin released after 30 min (10<sup>-2.8</sup> mmol/l) is smaller than method M2 (10<sup>-1.4</sup> mmol/l). This release can be correlated to the inhibition zone in the antibacterial test.

With prior plasma activation, the total amount and the rate of nisin released (during the first 15 min) are higher for both M1 and M2, probably due to higher wet pick-up rate. For M1, the concentration of nisin released in water increases slightly from 10<sup>-2.8</sup> to 10<sup>-2.5</sup> mmol/l with plasma treatment. After 15 min, no

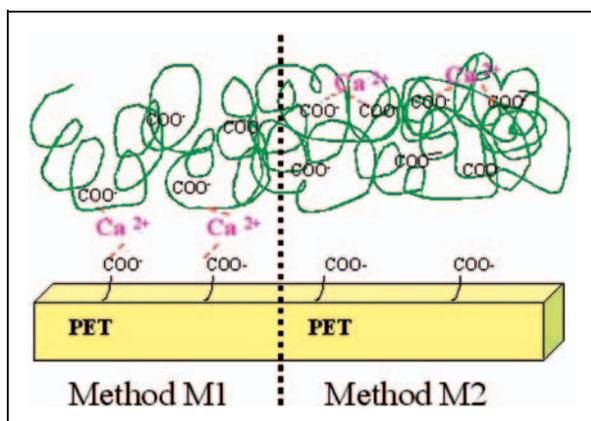
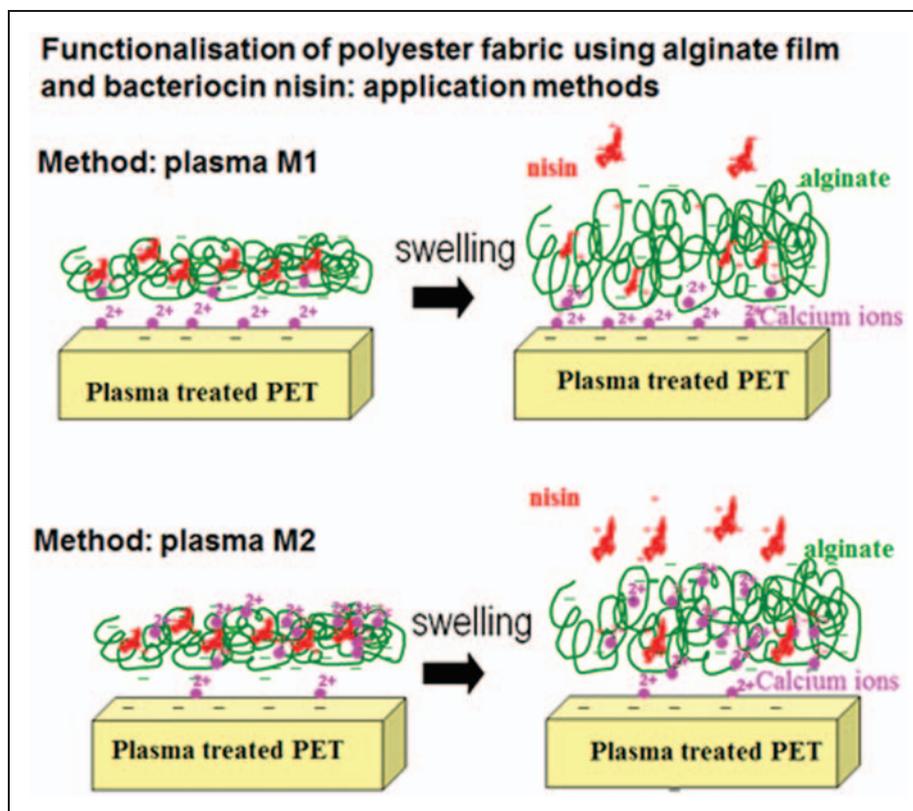
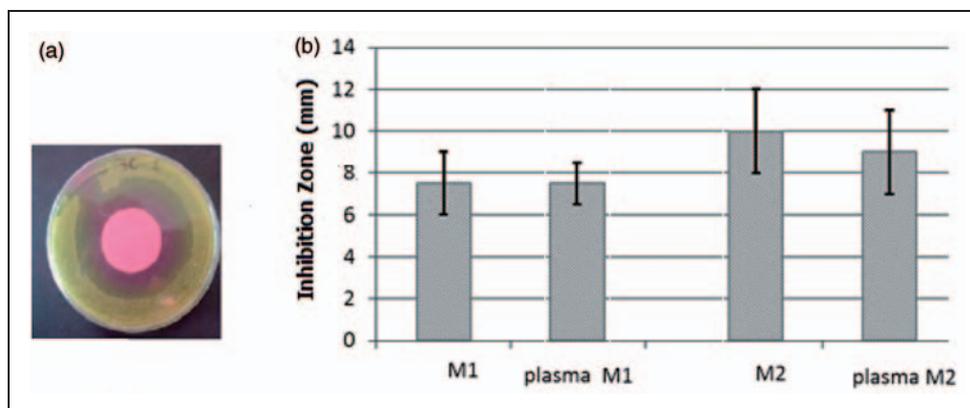


Figure 4. Organization of alginate polymer at PET fiber surface.



**Figure 5.** Nisin molecules entrapped in alginate films immobilized at the surface of plasma treated polyester fabric, before and after swelling in water.



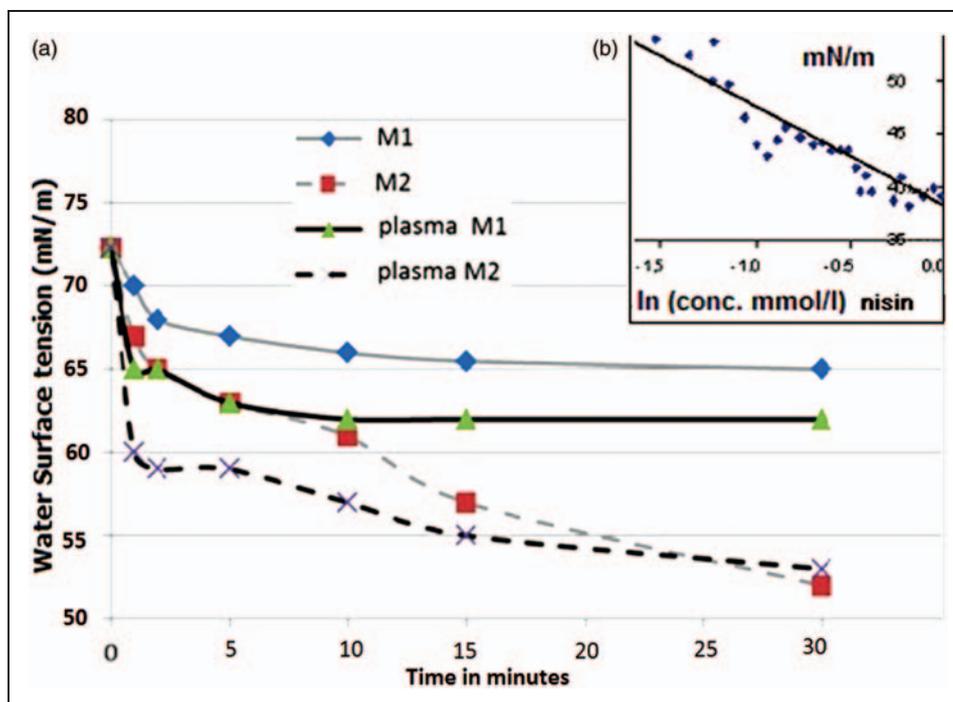
**Figure 6.** Antibacterial activity against *Staphylococcus* of PET fabrics functionalized with nisin entrapped in alginate film: (a) picture showing inhibition zone and (b) inhibition zones obtained for different methods.

further release of nisin is observed. The two treated fabrics (M1 and plasma M1) have the same inhibition zone in the antibacterial test, around 7.5 mm.

For M2, after 15 min, the concentration of nisin released without and with plasma treatment is  $10^{-1.4}$  and  $10^{-1.2}$ , respectively. Water surface tension decreases slowly with time of immersion. After

30 min, with plasma activation, nisin concentration in water reaches that of M2 without plasma.

**Discussion (part 2).** The nisin peptide has three cationic charges due to the three lysine amino acid groups in its chemical structure. These can form ionic bonds with free  $-COO^-$  groups of the alginate polymer. Nisin is



**Figure 7.** (a) Release kinetics of nisin entrapped in thin alginate film immobilized at the PET fabric surface using different methods. The quantity of nisin released was estimated using the calibration curve (b) showing the variation of water surface tension as a function of concentrations of nisin solution.

within the sodium alginate network which is deposited on the PET textile surface. Indeed, the release ability of nisin from the alginate film seems to depend on the quantity of  $\text{Ca}^{2+}$  ions deposited onto the alginate film or at the PET/alginate film interface. The smaller cations,  $\text{Ca}^{2+}$  ions, compete easily with the nisin molecules to form ionic bonds with the  $-\text{COO}-$  groups, making nisin molecules free. However, the free nisin molecules stay entrapped in the alginate gel film, and on drying, the free nisin stays entrapped in the dried alginate thin film. In wet or aqueous conditions (during the diffusion test in water or during the antibacterial test), the free nisin molecules can diffuse and leave the alginate-gel film. In the M1 method, most of the  $\text{Ca}^{2+}$  ions are deposited at the PET/alginate film interface, and are not available to free all nisin molecules in the alginate film, and so, no further release of nisin in water is observed after 15 min.

In M2 and plasma M2, higher quantity of  $\text{Ca}^{2+}$  ions (which are applied in the last stage) displace or free nisin more readily. Thus higher release of free nisin is observed with M2 compared to method M1. This would also explain the greater inhibition zone for the Chapman test for alginate films formed with method M2 compared to M1.

Moreover, though alginate film M2 may detach more readily from the PET surface, freeing some nisin molecules, it is less likely that this phenomenon may

occur during the antibacterial tests: the textile is not completely immersed in water, it is in contact with a water containing hydrogel-agar (wet conditions) which would barely induce the rupture of the alginate film at the PET surface. M2 film seems to provide a more long-term antibacterial effect, though its adherence to the PET fabric is not very high. M2 can be interesting method for functionalizing textiles, where durability of alginate film in water is not a prime importance, and where a long-term antibacterial effect is a more desired effect (e.g. disposable protective apparel or masks, or cheese packaging).

## Conclusion

This paper deals with the development of a thin alginate hydrogel film at the fiber surface of a porous textile, with little alteration in the porosity of the fibrous structure. The aim is to develop a porous structure based on woven PET textile bearing solid alginate film at the fiber surface, and capable of immobilizing a polypeptide such as nisin which may be easily released.

Alginate films were deposited at the fiber surface of a woven polyester fabric by padding process with or without a prior plasma activation of the PET textile structure.

The properties of the alginate films varied as a function of the alginate film formation method. The

presence of  $\text{Ca}^{2+}$  ions at the fiber/film interface and a prior surface activation with plasma treatment improved the adhesion properties of the film and hence its durability to wet treatment and mechanical strain.

When nisin was entrapped in the alginate film immobilized at the PET surface, all films showed antibacterial activity against *S. aureus*, with differences in the size of the inhibition zone depending on the alginate film formation method. Indeed by varying the alginate film formation conditions, the ease of diffusion of nisin molecules and the adhesion of alginate film changes, and so, the amount and the rate of release of the bioactive nisin can also be varied. The choice of film formation method will depend on the final use, and in particular whether a high film adherence or a more long term antibacterial effect is desired.

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