Electrospun nanofibres as a novel encapsulation vehicle for Felix O1 bacteriophage for new food packaging applications

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Abstract

Background & Objectives: Food contamination is one of the main issues in the food industry, and food packaging has been driven towards novel technologies to reduce bacterial contaminations. One of the possibilities is the development of antibacterial packaging by the encapsulation of bacteriophages, namely Felix O1, can provide protection against microbial pathogens like *Salmonella Enteritidis*.

This work aimed the encapsulation of Felix O1 into electrospun nanofibres to be used in biodegradable packaging as a controlling agent of *Salmonella Enteritidis* in food products.

Methods and Results: After the optimization of different electrospinning conditions (voltage, flow rate, polymer type, polymer concentration and type of solvent), polyvinyl alcohol (PVOH) with Felix O1 bacteriophage solution was electrospun on polyhydroxybutyrate/polyhydroxyvalerate film forming a layer composed by sub-micro nanofibres. The optimized conditions were: SM buffer solution of PVOH at 14% (w/v) at a flow rate 0.3 mL/h and applied voltage of 25 kV.

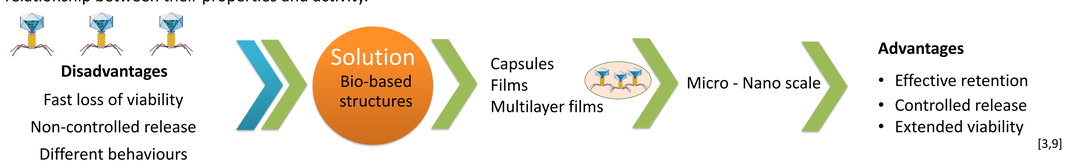
Conclusions: After the formation of nanofibres with a size around 100 nm (observed through Scanning Electron Microscopy), release tests in SM buffer for 1 h revealed a high bacteriophage viability (10^5-10^6), but still, there was a decrease of two log in the expected phage titre. DSC and TGA results revealed differences between the films and the films with nanofibres, showing the influence of the nanofibres in the system namely in the thermal behaviour. Results show that this new packaging system is promising for the development of active packaging using bacteriophages.

Significance and impact of the study: The encapsulation of bacteriophages through electrospinning shows high potential to be explored as a new feature in food packaging, as a possible solution for bacterial contamination in foods.

Introduction

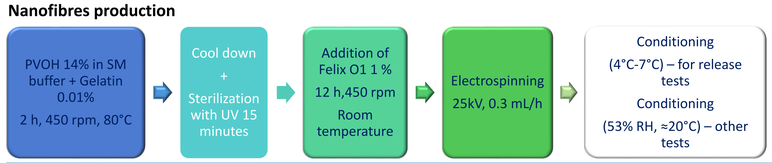
Food industry has on the production chain several stages that are continually changed in order to improve quality and safety of food products and develop products as a way to improve wellness of consumers, as a consequence of the increasing know-how in the food technologies. One of the major problems consists in microbial contamination leading to food spoilage and foodborne illnesses.[1,2]

The main objective of this work is the development and characterization of bio-based structures at micro- and nano scale for encapsulation of bacteriophages and the establishment of a relationship between their properties and activity.

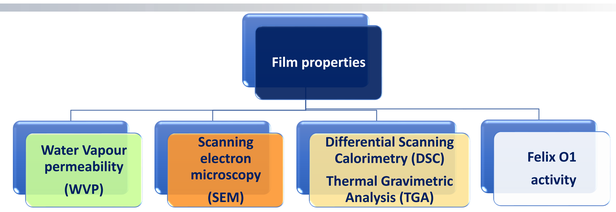


Methods

Nanofibres production



- Polyvinyl alcohol (PVOH), Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and Polylactic acid (PLA) were selected based on levels of toxicity and classification as GRAS type according to the acceptance by EFSA and FDA.
- Felix O1 bacteriophage from the *Myoviridae* family was selected based on their activity in *Salmonella*, and FDA approval to use in food products.
- Electrospinning was selected as the first method to produce PVOH fibres deposited in PHBV and PLA films.
- An experimental design was used to find out the best PVOH concentration, flow rate and voltage.



Results

Nanofibres optimization

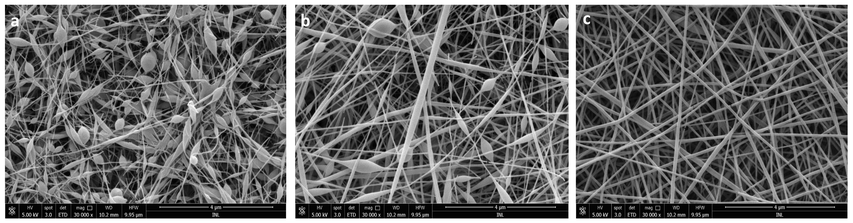


Figure 1. *Selected scanning electron microscopy microphotographs of PVOH fibres 8%, 0.3 mL/h, 25 kV (a), PVOH 11% 0.2 mL/h 20 kV (b), and PVOH 14% 0.3 mL/h 25 kV (c) formed using electrohydrodynamic process*

14 % of PVOH diluted in SM Buffer and 0.01% Gelatin was the selected formulation and 0.3 mL/h and 25 kV were the conditions used in the electrospinning process, regarding fibres homogeneity and processability.

Differential Scanning Calorimetry (DSC) and Thermal Gravimetric Analysis (TGA)

- PHBV + nanofibre presented lower melting temperatures compared to PHBV. Both samples had a two step melting behaviour probably due to the existence of crystals that recrystallize after melting forming more crystals that melt at higher temperatures.
- PHBV films presented 283.4 °C as degradation temperature while for PHBV films with nanofibres (PVOH 14%+SM Buffer+0.01% Gelatin + Felix O1) the highest degradation was around 274.0 °C but a second degradation temperature appeared at 290.5 °C.

Table 2. *Values of TGA decomposition temperature T_{d1} and T_{d2}, DSC melting temperature T_{m1} and T_{m2} and melting Enthalpy ΔH_{m1} for PHBV films and PHBV films with nanofibre*

Sample	T_{d1} (°C)	T_{d2} (°C)	T_{m1} (°C)	T_{m2} (°C)	ΔH_{m1} (J/g)
PHBV	283.4	-	141.5	153.9	9.4
PHBV+ nanofibre	274.0	290.5	137.2	145.0	7.5

Selection of matrix deposition

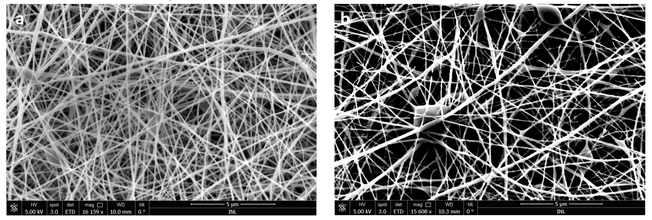
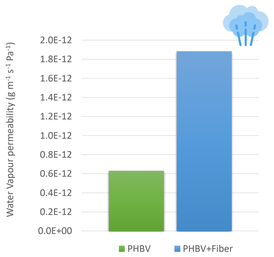


Figure 2. *Selected scanning electron microscopy microphotographs of PVOH 14% dissolved in SM buffer and 0.01% Gelatin with Felix O1, 0.3 mL/h 25 kV : in PHBV film (a) and PLA film (b) formed using electrohydrodynamic process*

- PHBV films allow to produce improved PVOH fibres comparing with PLA films.

Water Vapour permeability



- PHBV, WVP values are in accordance with the literature [1].
- WVP values increased from $6.3*10^{12}$ g m^{-1} s^{-1} Pa^{-1} (PHBV) to $1.9*10^{11}$ g m^{-1} s^{-1} Pa^{-1} (PHBV+nanofibre)
- PHBV+nanofibre results are related to the hydrophilic character of PVOH used to form the nanofibres that was in contact with the 100% of RH.

Nanofibres activity

Release tests in SM buffer for 1 h revealed a high bacteriophage viability (10^5-10^6), but still, there was a decrease of two log in phage titre (**Table 1**).

Table 1. *Values of Felix O1 activity in : PVOH+ SM Buffer + 0.01% Gelatin + 1% Felix O1 solution (a), PVOH+ SM Buffer+ 0.01% Gelatin+ 1% Felix O1 fibre predicted value (b), PVOH+ SM Buffer + 0.01% Gelatin + 1% Felix O1 fibre (c), PVOH +SM Buffer + 0.01% Gelatin fibre (Control) (d)*
** The values of (c) and (d) were measured after immersing the samples in SM buffer for 1h*

Sample	a	b	c*	d*
Felix O1 Activity (PFU/mL)	$4.2*10^9$	$1.4*10^8$	$2.2*10^6$	0
	$7.8*10^8$	$2.6*10^7$	$4.1*10^5$	0

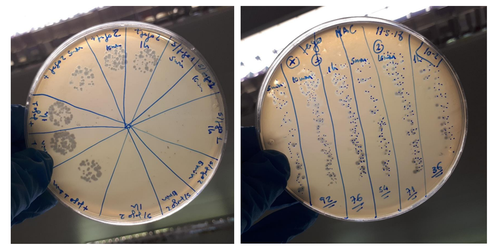


Figure 4. *Release tests of Felix O1 from PHBV+nanofibre into SM Buffer solution at 5, 10 and 60 minutes*

References

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Conclusions

PVOH nanofibres deposited in PHBV films allow the production of homogeneous fibres with a diameter around 100 nm.
Bacteriophages are able to be incorporated into PHBV+nanofibres through electrospinning and maintain a high activity 10^5- 10^6 PFU/mL .
Nanofibres presence results in higher WVP due to PVOH, also TGA and DSC results showed nanofibre influence in the thermal behaviour.
Further tests of moisture, solubility, mechanical properties and retention and release tests in liquid and solid simulants are important to understand the fibre performance.
These studies will promote the use of bacteriophages in food applications to ensure food safety and an increasing knowledge in new active systems where the information is still scarce.