

Electrospun Micro and Nanofibres of Olive Oil and Polyvinilic Alcohol

Gonzalez Silva, J.A¹, Olguin Fuentes, M.P¹, Santacruz Vázquez, C¹, Toxqui López, S², Laguna-Cortés, J.O³, Santacruz Vázquez, V^{1*}

¹Facultad de Ingeniería Química, Benemérita Universidad Autónoma de Puebla Av San Claudio, Ciudad Universitaria, Puebla, Pue México. C.P. 72570

²Facultad de Ingeniería, Benemérita Universidad Autónoma de Puebla Av San Claudio, Ciudad Universitaria, Puebla, Pue México C.P. 7257

³Departamento de Ciencias Básicas Tecnológico Nacional de México, Instituto Tecnológico de Puebla. C.P. México 72000

*Corresponding author: V. Santacruz Vázquez, Facultad de Ingeniería Química, Benemérita Universidad Autónoma de Puebla Av San Claudio, Ciudad Universitaria, Puebla, Pue México. C.P. 72570, México, E-mail: versanva@gmail.com

Abstract

Electrospun micro and nanofibres were obtained from an emulsion of Polyvinyl Alcohol (PVA) at a concentration of 10% and olive oil at 4 and 8% w / w. Scanning electron microscope images showed tubular, continuous fibers, whose diameters are dependent on the concentration of the PVA and olive oil. Fourier transform infrared spectroscopy indicated that the obtained fibers were composed of both components. The study demonstrated the potential to encapsulate liposoluble compounds in polymeric fibers using the electrospinning technique.

Keywords: Electrospinning; Fibers; Polyvinyl alcohol; Olive oil; Encapsulation

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Introduction

Electrospinning is a method of obtaining nanofibers^[1]. The assembly for the execution this technique consists of a capillary injector, through which a polymer solution is ejected, and a high voltage source that has two electrodes, one of which must be connected to the injector and the other directly to the collecting plate where the fibers are deposited^[2]. The technique can be set up horizontally or vertically (Figure 1).

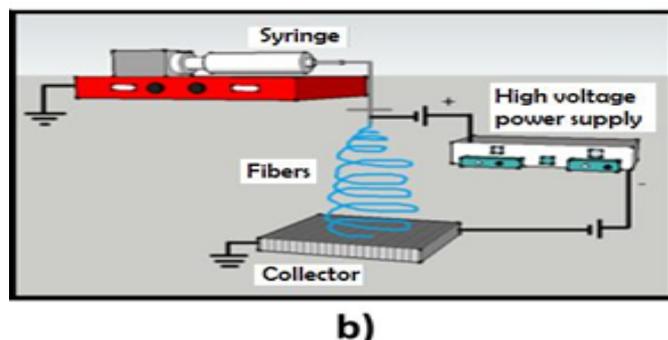
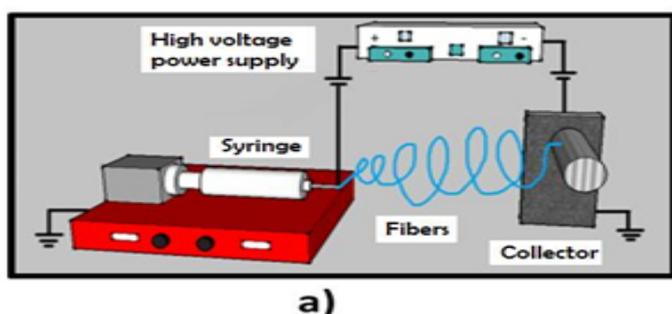


Figure 1: Installation scheme for horizontal electrospinning (a) vertical electrospinning (b)

The polymer must be diluted in any solvent or solvents that allow a complete dilution thereof in order to avoid clogging the capillary injector during the process, but at the same time promoting the production of homogeneous fibers. The polymers used in the electrospinning process are dielectric and in the presence of an electric field, can be considered as an arrangement of

microscopic electrical dipoles composed of positive and negative charges whose centers do not coincide and only change their position in response to strong external electric fields, allowing the solution stretch and form fibers^[3].

It is possible to increase the dielectric properties of the solution for example by adding solvents with high dielectric values^[4]; this favors the formation of fibers with fewer defects^[5]. When the solution has been introduced into the injector and the electric potential difference applied, charges are accumulated in the drop. This then lengthens to create a shape known as a Taylor cone. When the forces due to the electric field strength exceed the cohesive forces of the solution, a jet of polymer solution is elongated due to the electrical interactions between the molecular charges in the vicinity and begins a journey from the tip of the injector to the collector plate. Along the path of the fibers, the solvent evaporates and the fibers solidify upon arrival at the collector^[6]. There are numerous variables that may influence the properties and characteristics of the electrospun fibers and the control of these variables during execution of this process is of the utmost importance. The concentration of the solution is one of the parameters that effects the morphology of the fibers, since it influences the surface tension, solution viscosity and fiber diameter^[7,8]. Solution conductivity is another variable that affects fiber formation through the transport of charges^[1]. The addition of salts to the polymer solution increases the conductivity and consequently the electric force for the stretching of the polymer jet. This promotes a reduction in the diameter of the fibers^[9,10]. Another important parameter to be controlled is the potential difference; high voltages allow the fluid to be transported quicker, allowing fibers with larger diameters to be obtained^[11]. Furthermore, the variation of the distance between the injector and the collector also affects the morphology and diameter of the fibers. When working with a very large distance the electrospun fibers can break under their own weight, especially if they have small diameter. However, a minimum distance is required to allow the solution solvent to evaporate before reaching the collector plate, otherwise wet drops or fibers may form and, consequently, flattened or ribbon-like fibers may be obtained^[11,12].

Encapsulation

The aim of encapsulation is to protect the active components from the adverse conditions of the medium such as high temperature, the presence of light and oxygen^[13], pH change, presence of enzymes and other^[14], by the application of a polymer coating. The encapsulating agents are classified in various categories as waxes, proteins, carbohydrates and food grade polymers such as Polyvinyl Alcohol (PVA)^[15,16]. PVA is obtained by hydrolysis of polyvinyl acetate with fractions of molecular weights between 13,000 - 200,000 and whose physical properties, solution surface tension, rheological properties and water solubility depend on the degree of hydrolysis. PVA has the ability to form films and coatings that are highly resistant to traction, flexibility and have a low permeability to gases such as oxygen, carbon dioxide, and other vapors^[17]. Toxicological studies have found that this substance has the lowest absorption rate in the body; its oral intake is approved with an Acceptable Daily Intake (ADI) dose of 50 mg / kg body weight^[17,18]. Due to the chemical structure of the polyvinyl alcohol strong intermolecular interactions occur between the -OH groups and polar solvents.

Olive oil

Olive oil is composed of an oil fraction with 99% in the form of triglycerides and free fatty acids and presents a balance between omega-6 and omega-3 fatty acids. It is also a source of fat-soluble vitamins (A, D, E, K)^[19,20]. It is possible to develop polyunsaturated oil electrospun fibers that allow their physical, oxidative and chemical stability given that the majority of the oil encapsulation Processes include micro and nanocapsules. However, little is known about the development and incorporation of fibers. Therefore, the objective of this work is to develop a process for the production of encapsulated olive oil fibers using the electrospinning method, with Polyvinyl Alcohol (PVA) as the coating material.

Materials and Methods

Encapsulation of Olive Oil in Emulsions of Polyvinyl Alcohol (PVA)

For the encapsulation tests, extra virgin olive oil Huasco (Chile) was used, obtained by cold extraction. To obtain micro-fibers and nanofibers, Polyvinyl Alcohol solutions (PVA) were prepared, with a molecular weight Mw 13000 - 23000 (Meyer, USA, Cat. 5425), and distilled water (Meyer, USA, Cat. 0345) The concentrations of the PVA solutions were 8, 10, 12, 14 and 16% w / w. The polyvinyl alcohol was dispersed in distilled water, with a propeller stirrer at 600 ± 20 rpm and $80 \pm 1^\circ\text{C}$, for 30 minutes. Subsequently, when the temperature dropped to $25 \pm 1^\circ\text{C}$, the oil was incorporated to form an emulsion, which was subjected to mechanical stirring using a 250 W commercial immersion mixer for 5 minutes until homogenized.

Rheological and physicochemical analysis of emulsions

The viscosity of the emulsions was evaluated at a temperature of $25 \pm 1^\circ\text{C}$ by a Rheolab QC Anton Paar Rheometer (U.S.A.) using the DG24 concentric cylinder configuration and the Star Rheoplus Software^[21]. The Conductivity was determined (mS) as was the hydrogen potential (pH) with Conductronic model PC18 (Mexico) at Room temperature.

Electrospinning process

The PVA solutions and oil emulsions were poured into the syringe plunger of the electro-syringe equipment Prendo (SEV, Mexico) and different voltage conditions were tested, ranging from 20 to 0 kV, based on the report by Zhang et al.^[7] To obtain the fibers, the electrospinning equipment was operated under the following conditions: for the high voltage sources, 12 kV at the positive pole and 12 kV at the negative, an injection rate of 0.1 mL / h, a distance of 20 cm between the tip of the needle and the collector, rotational speed of the collector was 150 ± 5 rpm at a temperature of 24°C .

Morphological evaluation of fibers by SEM

The morphological and microstructural evaluation of the obtained fibers, was determined by means of a low vacuum SEM microscope, model JSM-5300 (U.S.A.) with a potential difference of 10 kV and image amplifications of 5000, 10000 and 20000X. To determine the average fiber size, twenty measurements of the diameter were made using ImageJ^[22].

Obtaining FTIR spectra of the fibers

The FTIR spectra permitted the identification of the principal characteristic spectral for each Components of the

fibers. For this test, the VERTEX model FTIR 70 (U.S.A.) was employed.

Determination of the water content of the fibres

The water content of the fibers was determined by drying 1 - 2 g of sample material in a convection oven (Thermoline U.S.A.) at 105 °C for 24 h and then calculating the mass loss^[23].

Determination of the water activity of the fibres

The water activity of the fibers was determined with an AQUA LAB (U.S.A.) hygrometer, calibrated previously, at a temperature of 25 ± 1°C.

Protective effect of PVA on the oxidation of olive oil

In order to evaluate the protection effect of the PVA encapsulating to olive oil, the films were irradiated in an ultraviolet light chamber, Prendo (SEV, Mexico), using a five lamp arrangement and light emission $\lambda = 254$ nm and exposure times of 30, 60 and 180 minutes. The L*, a* and b* color coordinates were evaluated on a Colorflex EZ45 colorimeter (Hunter Lab, USA). Subsequently, the color change of the fibers surface was determined using equation 1.

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad \text{Equation}$$

Statistical analysis

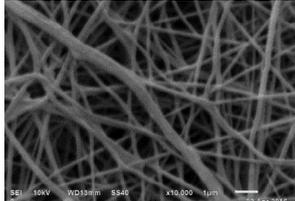
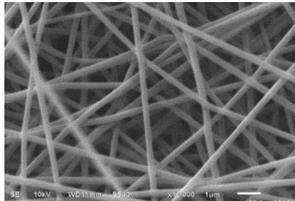
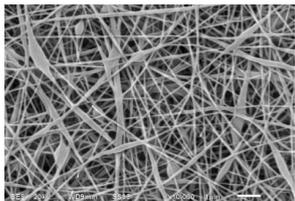
The results were analyzed by ANOVA, Tukey's multiple comparison procedure, using the SPSS system for Windows

(Version 15.0). All tests were carried out in triplicate; in the tables and figures the average values are reported. The differences were statistically accepted at $p < 0.005$.

Results and Discussion

The aqueous solutions of PVA presented values of electrical conductivity between 0.26 to 0.58 mS, which, according to the literature^[24], can be successfully electrospun. The pH of the solutions was determined to lie between 6.46 and 6.98. From a statistical point of view, these differences were significant given that the pH is an indicator of the free ions present in the solution. PVA is a polymer that is characterized by having negatively charged residual monomers OH⁻, which impart a polar nature. The analysis of the obtained experimental data suggests an inverse relation between the concentration of the PVA and the conductivity of the polymer solution; the higher the concentration of PVA, the lower the electrical conductivity and higher the viscosity. With respect to the viscosity, this is an important parameter for the formation of the fibers. It represents the resistance of the sample to flow into the injector. Average values of viscosity of 0.28 to 0.90 Pa.s are reported here, which are suitable for the formation of fibers from the polymer, as reported by Sousa et al.^[12] In (Table 1), the values of the viscosities, electrical conductivity and pH of the PVA solutions are shown.

Table 1: Values of the viscosity, electrical conductivity, pH of the PVA aqueous solutions and Their corresponding SEM micrographs for the fibers obtained.

Solution	Viscosity (Pa s)	Electrical Conductivity (mS)	pH	SEM micrographs for the fibers obtained Diameter fibers
PVA 8%	0.28 ± 0.02 ^a	0.58 ± 0.03 ^a	6.85 ± 0.13 ^a	 0.1206 ± 0.0210 μm ^a
PVA 10%	0.43 ± 0.03 ^b	0.49 ± 0.02 ^b	6.75 ± 0.11 ^b	 0.2592 ± 0.0180 μm ^b
PVA 12%	0.90 ± 0.03 ^c	0.26 ± 0.01 ^c	6.65 ± 0.10 ^b	 0.3599 ± 0.0370 μm ^c
PVA 14%	2.37 ± 0.08 ^d	0.20 ± 0.01 ^d	6.46 ± 0.15 ^c	It was not possible to obtain fibers
PVA 16%	5.21 ± 0.10 ^e	0.20 ± 0.01 ^d	6.20 ± 0.12 ^d	It was not possible to obtain fibers

^aMedia arithmetic of at least three replicates ± standard error. Values with the same letter within the columns are not significantly different according to the Tukey test ($P > 0.05$).

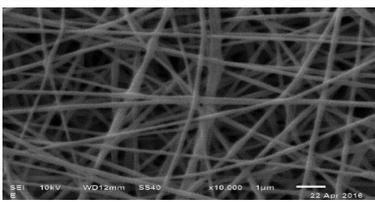
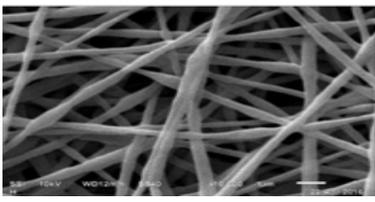
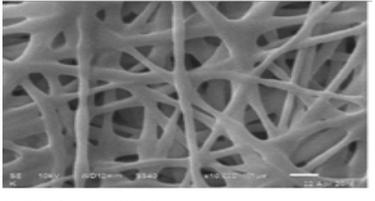
The Polyvinyl Alcohol (PVA) solutions at 8, 10 and 12% were electrospun and fiber formation was observed, whereas in PVA solutions at 14 and 16% no fibers were observed due to the high viscosity and low conductivity of the solutions. (Table 1) presents the images and the average diameters of the fibers obtained. The fibers at a concentration of 8% presented defects in their flattened morphologies which can be attributed to an excess in the moisture content in the formulation, i.e. During the process of electrospinning and transport of the fiber towards the collector the moisture content of the fiber did not evaporate out. This excess moisture content allows the fibers to deform on impact with the collector thereby forming a flattened shape. With respect to fibers at 10% concentration, it was possible to obtain homogeneous, non-deformed, cylindrical and regular fibers, indicating that the electrospinning conditions, as well as the PVA composition, were adequate. At 12% concentration, the experiments displayed aggregation defects due to the higher viscosity of the material and greater resistance of solution to flow during the passage through the injector. Such results were also reported by Fong et al^[25]. The average size of the PVA fibers was determined from the micrographs; it is observed that the increase in the average diameter of the fibers is related to the concentration of PVA in the solution. The 10% PVA concentration was selected to carry out the encapsulation of the olive oil, given the homogeneous and well defined characteristics of the fibers.

Characterization of the emulsions

In order to identify the oil composition in the PVA solution to be electrospun, different oil compositions were tested. They were identified as follows: PVA + 4 (A) corresponding

to a fiber composed of 10% polyvinyl alcohol with an addition of olive oil 4% (w/w), PVA + 8 (B) fiber composed of 10% polyvinyl alcohol with an addition of 8% (w/w) olive oil and PVA + 12 (C) 10% polyvinyl alcohol fiber with an addition of 12% (w/w) olive oil. The emulsions had an average diameter of $2.6 \pm 0.3 \mu\text{m}$. The physicochemical properties of the formulated emulsions are presented in (Table 2). The experimental results suggest an inverse relationship between the oil concentration and the conductivity and viscosity of the emulsion. The values of the conductivity of the emulsions varied according to the concentration of olive oil added. Although, as stated about, PVA is a polymer characterized by having negatively charged residual monomers reflected by the conductivity of the sample, during the addition of the olive oil, the conductivity was significantly reduced by the hydrophobic nature of the oil; the hydrophilic sites were diminished or masked. This phenomenon reduced the feasibility of creating nanofibers. Furthermore, there was an increase in the viscosity of the emulsion, which difficult the flow into the injector limiting the possibility of electrospinning. The same conditions for PVA fibers were also used for the electrospun emulsions. The micrographs of the fibers obtained are presented in (Table 2); in them it can be observed that the fibers that encapsulated the oil presented slight bumps. The composition of the PVA 10% + 4 and PVA 10% + 8 emulsions were suitable for obtain regular fibers, presenting a linear morphology with bulges due to the presence of olive oil. The measurement of diameter of fibers of PVA 10% + 4 showed that it was possible to obtain nanofibers, while PVA 10% + 8, only microfibers could be produced. The PVA 10% + 12 fibers had a non-uniform linear morphology, which implies that a high concentration of oil limits the possibility of fiber formation.

Table 2: Values of the viscosity, electrical conductivity and pH of the PVA encapsulated oil Aqueous solutions and their corresponding SEM micrographs for the fibers obtained.

Solution	Viscosity (Pa s)	Electrical Conductivity (mS)	pH	SEM micrographs for the Fibers obtained. Diameter fibers
PVA+4	0.37 ± 0.01^a	0.55 ± 0.01^a	7.28 ± 0.01^a	 $0.2038 \pm 0.0280 \mu\text{m}^a$
PVA+8	0.43 ± 0.01^b	0.52 ± 0.01^b	7.09 ± 0.01^b	 $0.4202 \pm 0.0100 \mu\text{m}^b$
PVA+12	0.56 ± 0.02^c	0.49 ± 0.01^c	6.80 ± 0.01^c	 $0.7041 \pm 0.0350 \mu\text{m}^c$

^aMedia arithmetic of at least three replicates \pm standard error. Values with the same letter within the columns Are not significantly different according to the Tukey test ($P > 0.05$).

Validation of the olive oil encapsulation in the fibers by FTIR

The symmetrical and asymmetric extension vibrations of the H-O-H bonds are located in the range 3490 - 3280 cm^{-1} and are associated with the water containing the fibers of PVA and oil. The band centered at 3007 cm^{-1} is assigned to the symmetrical extension vibrations of the linear chains =C-H of the alkenes present in the oil. This same vibration is observed in olive oil. On the other hand, the bands centered on 2957, 2922, 2873 and 2852 cm^{-1} are assigned to the asymmetric and symmetrical extension vibrations of C-H in lipids^[26,27]. With respect to the FTIR of PVA, a wide band belonging to the alkyl group ($\nu = 2850 - 3000 \text{ cm}^{-1}$), hydroxyl bands typical of alcohol -OH (OH-OH stretching band at $\nu = 3600 - 3650 \text{ cm}^{-1}$), and hydrogen bonded band ($\nu = 3200 - 3570 \text{ cm}^{-1}$), due to the intramolecular and intermolecular hydrogen bonds that develop between the PVA chains^[28] are observed. It is important to note that the analysis of the FTIR spectra made it possible to identify the olive oil encapsulated in the fibers and it was evident there was no interaction between the oil and PVA.

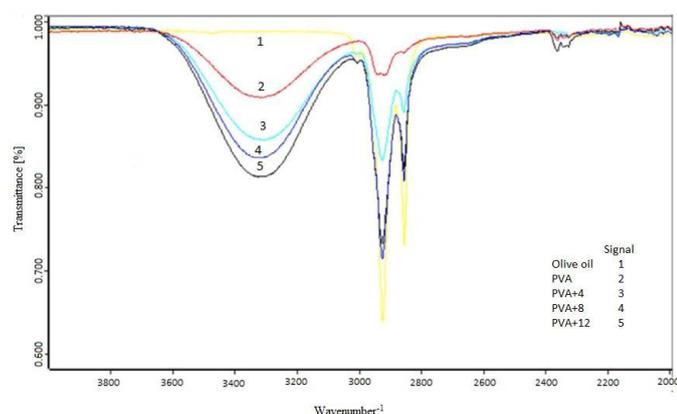


Figure 2 shows the FTIR spectra of PVA and PVA fibers with encapsulated oil and liquid oil.

Water activity and moisture content

The values obtained for the water activity (a_w) of the fibers were PVA, 0.386 ± 0.002 ; PVA + 4, 0.378 ± 0.002 ; PVA + 8, 0.385 ± 0.002 ; PVA + 12, 0.407 ± 0.002 . These values are very low which implies a low microbial growth in the fibers due to its low moisture content and low water activity during its storage. This behavior is reflected by the low moisture content of the oil fibers 0.104 ± 0.001 ; PVA + 4, 0.128 ± 0.002 ; PVA + 8, 0.144 ± 0.002 ; PVA + 12, $0.167 \pm 0.002 \text{ kg H}_2\text{O} / \text{kg dry solid}$.

Protective effect of PVA on the oxidation of olive oil

Irradiating the fibers with UV radiation indicated how photostable the fibers are. For exposure times of 30 and 60 minutes, no significant difference to the color of the fibers was observed. However, a significant change in the color of the surface of the fiber with exposure of 180 minutes was evident, turning yellowish color. The ΔE results are presented in (Table 3).

Table 3: Mean experimental values of the color change of UV irradiated fiber webs

Sample	ΔE		
	30 min	60 min	180 min
PVA	$0.10 \pm 0.02^{\text{a1}}$	$0.21 \pm 0.07^{\text{a2}}$	$0.92 \pm 0.08^{\text{a3}}$
PVA+4	$0.22 \pm 0.06^{\text{b1}}$	$0.41 \pm 0.08^{\text{b2}}$	$1.61 \pm 0.25^{\text{b3}}$
PVA+8	$0.32 \pm 0.08^{\text{c1}}$	$0.50 \pm 0.09^{\text{c2}}$	$4.10 \pm 0.45^{\text{c3}}$
PVA+12	$0.31 \pm 0.07^{\text{c1}}$	$0.61 \pm 0.10^{\text{d2}}$	$5.13 \pm 0.58^{\text{d3}}$

¹Media arithmetic of at least three replicates \pm standard error. Values with the same initial lowercase letter Within the columns are not significantly different according to the Tukey test ($P > 0.05$). Numeric values with The same superscript within the lines is not significantly different according to the Tukey test ($P > 0.05$).

The color difference (ΔE) was higher for an exposure time of 180 min in the four exposed fiber samples, being higher for PVA + 12 due to a higher concentration of encapsulated olive oil (Table 3). From these it can be concluded that the encapsulation process was efficient using a 60 minute exposure time. Regarding the values of L, a and b, it was observed that the changes in the parameters L and b were greater in magnitude. This means that, during their exposure to the UV radiation, the fibers presented an increase in the yellow tonality (b), a result of the initiation of oxidation reactions of the olive oil encapsulated in the fibers.

Conclusion

The electrospinning technique allowed the formation of PVA microfibers in which olive oil was encapsulated. The possibility of forming PVA nanofibers was identified, after which it was found that the morphology of the nano-fibers varied as a function of the concentration of PVA in the solution, presenting fibers with varying diameter of values between 0.1206 and 0.3599 μm . The electrospinning technique permitted the encapsulation of olive oil in PVA microfibers, with an average diameter of PVA + 4, $0.2038 \pm 0.0280 \mu\text{m}$; PVA + 8, $0.4202 \pm 0.0100 \mu\text{m}$ and PVA + 12, $0.7041 \pm 0.0350 \mu\text{m}$. The encapsulation of olive oil was verified by FTIR spectroscopy, highlighting the potential of the electrospinning technique through the identification of the double bonds corresponding to the unsaturation of olive oil. It was determined that the fibers obtained had low water content since 99% of the solvent was evaporated during electrospinning.

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Conflict of interest: The authors declare that there is no conflict of interest.

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