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# Polyvinyl alcohol and fluorescein electrospun fibers

# Fibras electrohiladas de alcohol polivinílico y fluoresceína

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**Abstract**

Electrospinning is a technique that allows obtaining new fibrous structures from synthetic or natural polymers for the development of materials used in the pharmaceutical and biomedical industries, among others. However, the low production rate of electrospinning has limited its industrial application, forcing the development of new injectors that allow higher productivity. In this work, a coaxial injector was designed to develop products encapsulated in polymeric fibers. For the demonstration of the encapsulation of one fiber included in another, fluorescein was used as internal compound in a polyvinyl alcohol solution, and polyvinyl alcohol was used as external fiber. It was obtained that the encapsulation process is possible by using this coaxial injector.

**Nanofibers, Coaxial electrospinning, Coaxial injector**

**Resumen**

El electrospinning es una técnica que permite obtener nuevas estructuras fibrosas a partir de polímeros sintéticos o naturales para el desarrollo de materiales utilizados en la industria farmacéutica y biomédica, entre otros. Sin embargo, la baja tasa de producción del electrospinning ha limitado su aplicación industrial, forzando al desarrollo de nuevos inyectores que permitan una mayor productividad. En este trabajo se diseño un inyector coaxial con el cual se desarrolló productos encapsulados en fibras poliméricas. Para la demostración de la encapsulación de una fibra incluida en otra se empleó la fluoresceína como compuesto interno en una solución de alcohol polivinílico, y como fibra externa se empleó el alcohol polivinílico. Se logró obtener que el proceso de encapsulación es posible mediante el uso de este inyector coaxial.

**Nanofibras, Electrohilado coaxial, Inyector coaxial**

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## Introduction

Currently, there are different methods for the manufacture of fibers and microfibers, including electrospinning or electrospinning. This technique was observed by Rayleigh in 1897, who evaluated the effect of inducing electrical charges in water jets, as well as the asymmetric instability of the jet flow. The electrospinning process allows the formation of fibers at micro and nanometer scales in a simple way, generating important advantages over other fiber production methods. However, there are several configurations of electrospinners, horizontal and vertical, with injector, simple, carousel or coaxial injection. The latter configuration has been of great interest in recent years.

This interest is not accidental, since the quality of the fiber or microfiber will depend to a large extent on the proper functioning of this system, which in turn will determine such important parameters as the encapsulation and release of an active substance, especially in view of the ever-increasing requirements in the pharmaceutical field. But this requires a thorough understanding of all the phenomena involved in the coaxial injection process itself.

Undoubtedly, one of the most critical parts in this process is the composition of the solution, and more specifically, the diameter of the internal and external capillary tubes used in the coaxial injection system. From the geometry of these will depend the characteristics of the fibers that can be obtained, and from the flow conditions just at the exit of the orifice will depend the behavior of the Taylor cone. Therefore, the objective of this work was to obtain electrospun fibers of polyvinyl alcohol and fluorescein using a coaxial injector.

Only until a few years ago due to the demand for materials with nanometer scale dimensions, the electrospinning technique has become a more attractive process thanks to the ability to transform a wide range of materials in the form of nanofibers at low cost and with relative simplicity.

The electrospinning equipment consists of a capillary through which the polymer solution is expelled; a high voltage source that has two electrodes, which must be connected one to the outlet of the solution and the other directly to the collector plate, conductive metal sheet or rotating mandrel, among others. To start the process, the polymer must be diluted in the solvent or solvents that allow dilution to obtain homogeneous fibers.

The polymers used are dielectric, and in the presence of an electric field can be considered as an arrangement of microscopic electric dipoles composed of positive and negative charges whose centers do not coincide perfectly (Dekker, 1959), remaining in place by the action of atomic and molecular forces, and can only change their position slightly in response to strong external electric fields, which explains why the stretching of the solution occurs in the process (Fano, 1987). Sometimes to increase the dielectric properties of the solution, some solvents with high dielectric constants are added (Lee, Kim, Khil & Ra, 2003), this favors the formation of fibers with less defective "droplet" structures and reduced diameters (Son, Youk, Lee, & Park, 2004).

Once the solution is in the injector, the application of high voltage is initiated, the charges accumulate promoting the formation of a droplet at the tip of the capillary and as the electric field strength increases, the droplet elongates to create a conical shape known as a Taylor cone. The electric field strength overcomes the cohesive forces of the solution, in most cases dominated by surface tension, as a jet of polymer solution begins a journey from the tip of the capillary to the collector plate; on its journey, the polymer solution jet is elongated due to electrostatic interactions between charges near segments of the same jet, meanwhile, the solvent evaporates, finally, the fibers solidify upon arrival at the collector plate (Chandran, Ravichandran, Chandran, Chemmanda, & Chandarshekar, 2016). There are several operational parameters that are intimately related to the properties and characteristics of the electrospun fibers so their control is indispensable.

Among the most important variables in the electrospinning process are:

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Concentration of the solution, surface tension, conductivity of the solution, dielectric properties of the solvent, while the external variables of the electrospinning process are voltage, output flow and distance between the needle tip and the collector, to mention the most important ones (Moreno-Cortez et al., 2015).

## Types of injector

There are several configurations for the electrospinning process, and they are the horizontal and vertical configuration; referring to the position of the injector and collector that can be horizontal and vertical respectively. Similarly of the injectors present modifications in their configuration, among it is the single injector, needle injector or also called capillary, coaxial injector and multi-injector (Yao, Chang, Ahmad, & Li, 2016).

The needle injector is the most commonly used configuration according to the literature and was the first injector designed for the electrospinning process (Huang, Zhang, Kotaki, & Ramakrishna, 2003). It consists of an embolus or syringe with an electrically charged capillary attached, which serves as a duct for feeding the fluid to be electrospun. Unfortunately it presents disadvantages such as: low feed flow, impossibility to obtain fibers from immiscible materials, low encapsulation capacity (Moreno-Cortez et al., 2016; Tang et al., 2016; Vyslouzilova et al., 2017).

The coaxial injector is a modification of the conventional electrospinning process that involves the arrangement of multiple feed systems for simultaneous electrospinning. Generally, a matrix arrangement of needle injectors is employed (Figure 1), allowing the injection of an internal solution into an external one, this to produce continuous coated or hollow nanofibers.

The coaxial electrospinning configuration involves a core-shell nozzle attached to a dual-compartment syringe (Yarin, 2011) that consists of coupling an inner needle or capillary into the concentric outer needle, which is connected to the core and shell solvent reservoirs, respectively. Figure 1 presents the schematic for Taylor cone formation in a coaxial injector, noting that the conductivity and properties of the outer or shell polymer are important for fiber formation.


**Figure 1** Schematic illustrating Taylor cone formation in the coaxial injector. A) Surface charges b) Shell formation in the droplet c) Taylor cone for shell formation
*(Derived from Hao R, Yuan JY, Peng Q, 2006)*

The application of an electrostatic field in the coaxial injector results in the formation of the composite Taylor cone consisting of the core solvent surrounded by an envelope solvent, as the inner fluid is pushed inward and embedded within the envelope fluid to give an outer fluid.

Since the pioneering work on this topic was published in the early 21st century (Sun et al., 2003, Loscertales et al., 2002, Zhang et al., 2004), modification of the original injector has been achieved for better feasibility and applicability. The importance of this injector lies in the possibility of producing biphasic fibers from fluids with different physical and chemical properties, allowing the formation of fibers of electrospinnable materials without mixing, in the form of polymer: polymer/organic compound and polymer/inorganic compound. This technique allows obtaining sophisticated conduits for drug delivery, energy storage and sensors (Zhang et al., 2015); It optimizes processes as is the case of nanotube fabrication, in which the vapor deposition phase is eliminated.

This coaxial injector allows obtaining materials with innovative nanoscale architecture (branched, thread-in-tube, multichannel, porous) and corresponding hybrids (Wu et al., 2013), focused for the design of technological applications in sensors, engineered fabrics, drug delivery systems and nanoelectronics (Greiner and Wendorff, 2007; Bandyopadhyay-Ghosh et al., 2015). Some materials such as metal salts and enzymes possess unique properties at the nanoscale level; However, their capabilities to form nanofibers are limited by a number of factors including molecular weight, solubility, low solvent conductivity and high surface tension and thus the use of coaxial injector allows forming a protective coating that encapsulates the desired compound in the core.

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For example, Teflon cannot be electrospun due to a low dielectric constant that prevents sufficient charging of the polymer, but it possesses hydrophobic properties necessary for the textile, construction and automotive industries, among others. Therefore, coaxial electrospinning was employed to take advantage of these properties of the polymer, and its encapsulation in poly (ε-caprolactone) (PCL) (Wu et al., 2013).

Chen et al. (2010) fabricated a nanotube structure from two fully miscible solvents through a set of three coaxial capillaries employing a chemically inert fluid between them as a separator.

As for the biomedical area, the application of coaxial electrospinning has made possible the safe fabrication of nanocomponents and microstructures of highly unstable materials, such as enzymes, that would otherwise decompose in reactive environments (Gu et al., 2013; Davis and Leach, 2011). It has been reported that this device allows an increase in the encapsulation process of active compound (Garcia-Moreno et al., 2016) and uniformity in the fibers, greater protection of the encapsulated actives (Yao, Chang, Ahmad, & Li, 2016), however this injector is a challenge for the materials designer since this configuration presents a different problematic and depends on several parameters such as viscosity, miscibility, surface tension of the solutions to be injected.

**Encapsulation**

Encapsulation is a process used for the preservation or protection of numerous commercial ingredients, not only food, but also pharmaceuticals, chemicals and cosmetics. The pharmaceutical and food industry applies encapsulation for several reasons: to stabilize the active agent, to control the release of the encapsulated material (rate and form of release), and to separate reactive or incompatible components. Encapsulation processes have been developed as a response to the loss of viability of those active components present and consist of the protection of these materials by covering them with a carrier or encapsulating agent.

Whose objective is to protect the active component from environmental conditions such as temperature, light, oxygen, pH, enzymes, presence of other nutrients (Chen, 2007), which diminish the beneficial effect of the active component for which it is intended (Pszezola, 2005). The quality of the encapsulates, i.e. their efficiency in protection and controlled release, depends on several factors, including: the operating conditions during production (temperature, pH, pressure and humidity) and the handling of these particles, as well as the composition and structure of the materials used (Fuchs et al., 2006).

Flavor retention is determined by factors related to the chemical nature of the active agent, including its molecular weight, chemical functionality, polarity and relative volatility, the properties and nature of the encapsulating material or carrier agent, as well as the parameters of the encapsulation process. Currently there is a wide variety of encapsulating materials and active agents including waxes and lipids, proteins such as gelatins, whey proteins, zein, soy proteins, gluten, among others, carbohydrates such as starches, maltodextrins, chitosan and biopolymers such as polypropylene, polyvinyl acetate, polystyrene, polybutadiene and polyvinyl alcohol.

The following is brief information on polyvinyl alcohol, since it is a polymer used as an encapsulant, but it is also reported in a number of studies with application in electrospinning. PVA is obtained by hydrolysis of polyvinyl acetate and its commercial presentations have between 80 and 100% hydrolysis and molecular weight ranging from 13,000-200,000.

The degree of hydrolysis of PVA affects its physical properties such as interfacial tension, biocompatibility, rheological properties of solution and water solubility (Abd El-aziz, El-Maghraby, & Taha, 2016). In the pharmaceutical industry it is widely used to coat tablets and capsules while in the cosmetology industry it is used to bind product components. In toxicological studies it has been found that the degree of absorption of this substance by the body is minimal so its oral consumption is approved.

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According to the background presented, the objective of this project is the development of novel materials using the electrospinning technique and coaxial injector to obtain coaxial fibers using polyvinyl alcohol (PVA) and fluorescein.

## Methodology

### Obtaining fibers using the single injector

The polyvinyl alcohol and fluorescein solutions to obtain the fibers were worked with voltages of 5 and 15 KV in each source, with a rotation speed of 100 RPM. Obtaining fibers of 90 - 110 nm based on what is reported in the literature.

### Obtaining fibers using coaxial injector

The polyvinyl alcohol and fluorescein solutions were fed independently into the coaxial injector ducts for electrospinning, initially they were tested with the conditions shown in Table 1.

| Injector type | Injector-collector distance (cm) | Voltage (kV) | Fluorescein feed flow (mL/hr) | PVA feed flow rate (mL/hr) |
|---|---|---|---|---|
| Single injector | 15 | 5-15 | 0.5 | 1 |
| Coaxial injector | 15 | 5-15 | 0.5 | 1 |

**Table 1** Electrospinning conditions for coaxial injector

### Solution preparation and electrospinning

For the encapsulation tests, a solution of 87% hydrolyzed technical grade high-density polyvinyl alcohol (Meyer, USA, Cat. 5425) was used. For the encapsulation tests, an 8% solution of PVA with fluorescein (Aldrich, USA, Cat. 2456) was used as the active compound.

The characterization of the PVA and fluorescein solutions was carried out with the aid of Conductronic model PC18® equipment at room temperature, determining the following values of conductivity (mS) and hydrogen potential (pH).

The evaluation of the FTIR spectra made it possible to identify the main reflections of the spectra of each of the fibers, using a database provided by the supplier to identify the main functional groups of the molecule. The VERTEX 70® model FTIR equipment was used for this test.

The morphological and microstructural evaluation of the fibers was determined through a Scanning Electron Microscopy equipment, which worked with a potential difference of 10KV, detecting backscattered electrons and amplifications of 500, 1000 and 5000X were made. The SEM images obtained were analyzed using ImageJ® software.

## Results and discussion

The coaxial injector was constructed by two injectors (see Figure 2) that are located one inside the other on the same side of the physical structure of the electrospinning system, and in a horizontal position with respect to the collector. This concept consists of an external injector whose function is to encapsulate the whose function is to encapsulate the active component (Fluorescein) in the nanofiber generated during the electrospinning process; it is proposed to have the same length of the internal injector for a better encapsulation, since, otherwise, if it has a longer or shorter length, the nanofibers will not have the necessary conditions and deformations will occur in them.

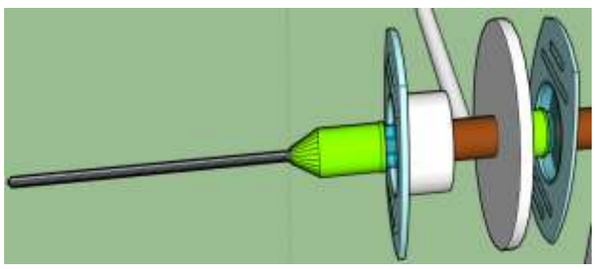

**Figure 2** Drawing of the coaxial injection system

This coaxial injection system has a vertical support according to the target specifications that were initially proposed in order to provide greater stability to the collection system. Thus, the function of this system is to encapsulate the aforementioned components, together with the electrospinning equipment by means of carbon brushes to the transverse axis the grounding of the electric field required during the electrospinning process. Finally, the last piece that composes this coaxial injection system are the containers of the solutions to be electrospun, which store the same amount of solution volume in order to increase the stability of the injection system.

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## Characterization of the solutions

Analyzing the values obtained for pH and citing the literature on the importance of this parameter, it is commented that pH is a significant variable for the process of nanofiber formation and electrospinning. The pH is a measure of the concentration of dispersed ions present in the chemical structure of the emulsion components. For the case of biopolymers pH is a parameter that controls their physical properties, specifically for the PVA solution used in this study and given its polyhydroxylated nature dipole-dipole bonds, induced dipole-dipole and dispersion forces are present.

Hydrogen bridges are a special type of dipole-dipole interaction between the hydrogen atom of a polar bond, such as N-H, O-H or F-H, and a highly electronegative atom such as fluorine (F), oxygen (O) or nitrogen (N) and exert a constant interaction with H atoms. These bonds are much stronger than Van der Waals bonds and, although they are weaker than covalent bonds, when taking into consideration a high number of them between polymer chains, they result in higher attractive forces in the polymers that present them.

Table 2 shows the viscosity, conductivity and pH values of the solutions used for the electrospinning process.

| Solution | Electrical conductivity (mS) | pH |
|---|---|---|
| PVA92%-FLUORESCEIN 8%. | 0.26 ± 0.01 | 6.65 ± 0.01 |
| 10% PVA solution | 0.48 ± 0.01 | 6.75 ± 0.01 |

**Table 2** Physicochemical Properties of Fluorescein and PVA Solutions

Regarding the analysis of the conductivity data of the PVA and fluorescein solutions, the electrical conductivity values were in the range 0.26 to 0.49 mS and these values were found to be a function of the PVA concentration, since the fluorescein solution is added in a small amount and therefore does not significantly affect the viscosity of the solution. Similarly, the pH of the solutions was determined and it can be observed that the lower the concentration of PVA, the value is slightly higher than the other sample.

The effect of PVA concentration on the conductivity values is due to the polymeric structure, which is characterized by having residual monomers positively charged by the OH- radical, which imparts a polar nature that is reflected in the conductivity of the sample. The increased conductivity of the solution accentuates the ability to become electrically charged, facilitating the stretching of the nanofiber during its formation.

The optimum conditions for obtaining electrospun fibers of polyvinyl alcohol and fluorescein using a coaxial injector were found. The ideal flow rate for both polymers was 3.5 mL h-1 in the coaxial electrospinning system in which. A voltage and a distance between the needle and the collector plate of 15 kV and 15 cm, respectively, were used, as shown in Table 3.

| Parameter |
|---|
| Amount of polyvinyl alcohol: 0.5 g |
| Solution flow rate: 3.5 mL h-1 |
| Applied voltage: 15 kV |
| Distance between needle tip and manifold: 15 centimeters |
| Final volume of each polymer solution: 2 mL |

**Table 3** Optimal conditions for obtaining fibers in a coaxial injector

The progressive decrease in the concentration of PVA in the solution generates a notable decrease in viscosity. As well as a reduction in the concentration and surfactant power of the solution.

## Morphology by scanning electron microscopy

To characterize the morphology of the fibers, an SEM study was carried out. The micrographs are shown in Figure 3. The images indicate a uniform and smooth appearance, with no appearance of beads or droplets in the formed mat.

In photomicrographs shown in Figure 3 it is possible to determine that the PVA and fluorescein solution allowed an adequate electrospinning, since no large clusters of material are present in the web.

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According to the results found using ImageJ® software, the 10% PVA solution allowed the formation of the fluorescein fibers, this behavior is believed to be attributable to the concentration of amino acids with residual OH charge to the negatively charged collector and the correct evaporation of the solvent in its path.

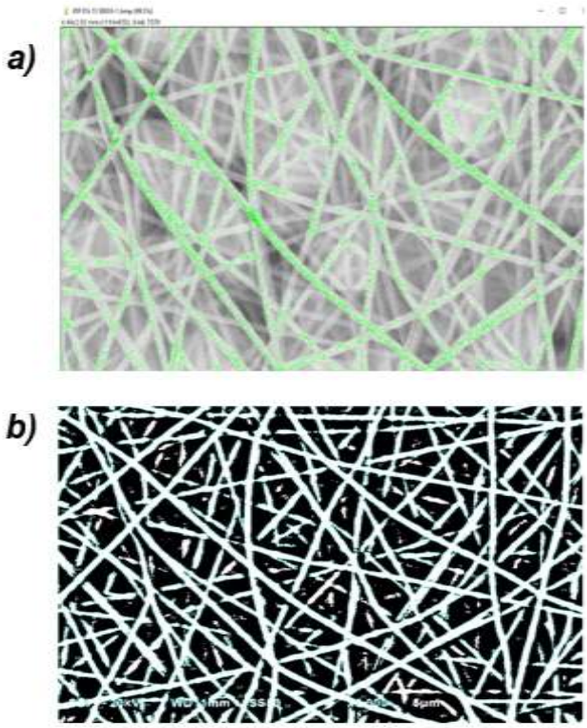


**Figure 3** Micrographs of PVA 10% - Fluorescein 90% in ImageJ ® a) 5000x and b) 5000x negative filter

## Component analysis by (FTIR); Fourier Transform Infrared Spectroscopy

In order to validate the presence of fluorescein incorporated in the fibers, the Fourier Transform Infrared Spectroscopy technique was used to identify the functional groups representative of the molecules that make up the fibers. Since the molecular structures of PVA and fluorescein are complex, the spectra of the pure substances were compared with the spectra of the different solutions. Figure 4 shows the ATR-FTIR spectra of the PVA fibers with fluorescein in addition to the PVA solution.

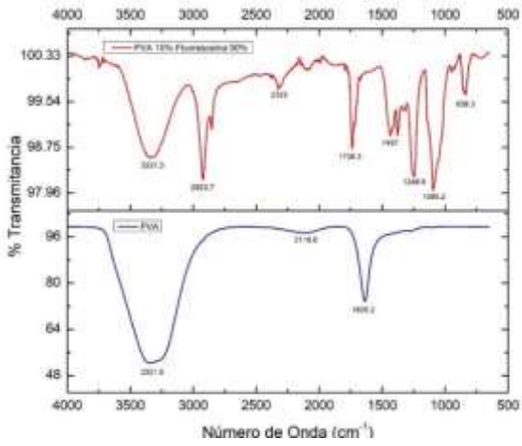


**Figure 4** Infrared spectra. a) PVA 10% - Fluorescein 90%, b) PVA

In the PVA solution, vibrations of symmetric and asymmetric extensions of the H-O-H bonds can be seen in the range 3490-3280 $cm^{-1}$ associated with the water containing nanofibers. The peaks between 2962 $cm^{-1}$ and 2972 $cm^{-1}$ correspond to the CH and $CH_2$ groups. Likewise, a peak of high intensity at 1738.3 $cm^{-1}$ corresponding to the C=O group strain of the residual acetate groups is also observed.

The presence of aldehyde-type compounds was also confirmed, giving rise to a band centered at 2325 $cm^{-1}$, assigned to C=O stretching vibrations; a band of strong intensity at 1738.3 cm-1 is also distinguished, which is assigned to C=O vibrations in aliphatic aldehyde chains.

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## Conclusions

In this section, the main achievements will be presented, emphasizing the most relevant ones for the objectives that were intended to be fulfilled; on the one hand, the development and testing of the coaxial injection system; and on the other hand, the use of this system to obtain polyvinyl alcohol and fluorescein electrospun fibers.

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The optimum conditions for obtaining polyvinyl alcohol and fluorescein electrospun fibers in a coaxial electrospinning system were determined. This is an efficient method for the fabrication of microfibers that allows adjusting the desired execution variables to have a better performance at the time of producing the microfibers. The electrospinning or electrospinning technique allowed the formation of PVA nanofibers in which the encapsulation of the orange essential oil extract was possible.

Considering that the PVA 90% - Fluorescein 10% fibers were the fibers that presented the best characteristics using a coaxial injector with capillaries with internal diameter of 0.3 mm and external diameter of 0.7 mm both with the same length, it is concluded that the electrospinning conditions were: Voltage 15 KV for the positive pole, voltage of 5 KV for the negative pole, collector speed of 100±3 rpm, distance between injector and collector of 15 cm.

The morphology of the nanofibers obtained varied as a function of the PVA concentration in the emulsion, presenting fibers with different diameters ranging from 0.0852 to 0.2936 µm. The fluorescein encapsulation was verified by FTIR spectroscopy, highlighting the potential of the electrospinning technique through the identification of the double bonds corresponding to the fluorescein unsaturation. According to the experimental results it can be concluded that in order to carry out the electrospinning process it is necessary to identify the types of residual charges of the polymeric molecule and their concentration.

The properties of the fibers that are developed by means of the electrospinning technique allow their use in the pharmaceutical area where controlled release of drugs is necessary in which the timely delivery of medicines is allowed.

In recent years, new ways of assembling the system have been investigated in order to provide new features, enhance the characteristics of the developed fibers and allow the encapsulation or electrospinning of materials that did not have the necessary electrical properties and therefore could not be subjected to the process.

These new adjustments have made it possible to process new materials, encapsulate a wide variety of drugs and maintain a better control of drug release.

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