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Design and automation of an electrospinning system to prepare micro and nanofibers. Case study: elaboration of polymeric micro and nanofibers for vaginal drug delivery

Diseño y automatización de un sistema de electrohilado para la preparación de micro y nanofibras. Estudio de caso: Elaboración de micro y nanofibras poliméricas de administración vaginal

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Abstract

In the present investigation was optimized and automated a prototype of an electrospinning system. In addition, the methodology for preparing the polymeric film with polycaprolactone micro and nanofibers (PCL) loaded with Neem extract was optimized as a proposal for the treatment of cervical cancer. Also, a UV-VIS spectrophotometric method was developed for the quantification of Neem extract encapsulated in PCL polymeric nanofibers through the formation of a colorimetric complex with FeCl3. The wavelength used to quantify the Neem extract was 423 nm. The prototype built allowed the formation of nanofibers loaded with Neem extract with a diameter of 22-71 nm in diameter. The encapsulation efficiency of the Neem extract was 78.4%.

Electrospinning, Cervicouterine cancer, Polymeric membrane

Resumen

En el presente trabajo se optimizó y automatizó un prototipo de sistema de electrohilado. El sistema de electrohilado, nos permitió preparar una membrana polimérica no tejida con micro y nanofibras de policaprolactona (PCL) cargadas con extracto Neem como alternativa para el tratamiento de cáncer cervicouterino. Además, se desarrolló un método espectrofotométrico UV-VIS para la cuantificación de extracto de Neem encapsulado en las nanofibras poliméricas de PCL por medio de la formación de un complejo colorimétrico con FeCl3. La longitud de onda utilizada para cuantificar el extracto de Neem fue de 423 nm. El prototipo construido permitió la formación de nanofibras cargadas con extracto de Neem con un diámetro de 22-71 nm. La eficiencia de encapsulación del extracto de Neem fue de 78.4%.

Electrohilado, Cáncer cervicouterino, Membrana polimérica

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Introduction

Cervical cancer is a health problem with a high mortality rate. Specifically, Mexico occupies the second place in cases, behind breast cancer. Its high incidence is related to ignorance and low culture of prevention, since this cancer is detectable, and if discovered in a timely manner, the chances of curing it are high.

According to the World Health Organization (WHO), there are more than 6 million cases of cancer in women; 57.2% of these cases occur in developing countries. In Mexico, cervical cancer is the second leading cause of cancer death in women. Annually, there is an estimated occurrence of 13,960 cases in women, with an incidence of 23.3 cases per 100,000 women (CNEGSR, 2016). Cervical cancer is multicausal and is due to the association of different risk factors. One of the main causes is human papillomavirus (HPV) infection. Co-infections of HPV with other sexually transmitted infectious agents, such as *Chlamydia trachomatis*, herpes simplex virus type 2 (HSV-2) and human immunodeficiency virus (HIV) possibly condition a synergistic effect that increases the chances of cellular alterations leading to the development of a neoplasm. *Chlamydia trachomatis* infection and marginally HVS-2 favor the entry and persistence of multiple HPV types, leading to viral integration, inhibition of apoptosis, overexpression of E6/E7 oncogenes and cellular transformation (Hernández-Hernández *et al*., 2015).

In addition, some of the causes of the presence of cancer are aggravated or originate from other vaginal infections. As of 2014 in Mexico, during reproductive age 75% of women experience at least one episode of vulvovaginal candidiasis. Approximately 6 to 55% of healthy women are asymptomatic carriers (Solís-Árias *et al*., 2014). Vulvovaginal candidiasis is a very common infection affecting at least 50% of women once in their lifetime; it accounts for 25% of total vaginal infections. Recurrences are frequent and it has been estimated that 50% of cases will present a second episode after the first clinical picture (Martinez-Perez *et al*., 2018). There are several cytotoxic drugs used in the treatment of cervical cancer, which present many adverse effects that are derived from the use of high doses and/or due to the lack of specificity in the therapeutic target.

Nanotechnology offers a series of opportunities for pharmaceutical innovation, by exploring new routes of administration that are less aggressive and more comfortable for the patient through the development of different therapeutic nanosystems, nanofibers, nanoparticles, nanocapsules, etc. Offering the possibility of significantly improving treatment schemes with a modified release of active molecules. The advantages of nanosystems such as: protection of labile drugs, controlled release, modulation of adhesion and/or penetration to vaginal mucus; offer a great opportunity for their application in vaginal drug delivery (Nunes *et al*., 2021). Nanofibers are an example of these nanosystems, generally possessing a diameter of less than 1000 nm, reaching even kilometer lengths (Zhang *et al*., 2018). Currently, their interest and research is increasing because they have the following advantages: greater surface area that allows greater interpenetration; high bioadhesive strength, which allows longer contact time and controlled release of active ingredients; easier and more comfortable administration for patients (Machado *et al*., 2016).

There are different techniques for obtaining nanofibers, which are limited by the type of material, type of solvents, manufacturing times and low process efficiency. For example, by controlling process factors such as applied voltage or flow rate of the solutions, it is possible to control nanofiber characteristics such as porosity, specific surface area, mechanical strength and morphology (Sanabria-Romero F., 2022). The electrospinning process is simple and has the following stages: a polymeric solution is contained in an injection syringe, the metal tip of the injection system is connected to a high voltage source (5-50kV), when the electric field is able to overcome the surface tension of the polymeric solution droplet deforming it to a conical "Taylor cone" tip, the polymeric solution starts to flow as a distorted jet which is attracted and electrically deposited on a collector plate as solid fibers, forming a polymeric membrane on the collector (Figure 1) (Velasco-Baraza *et al*., 2016).

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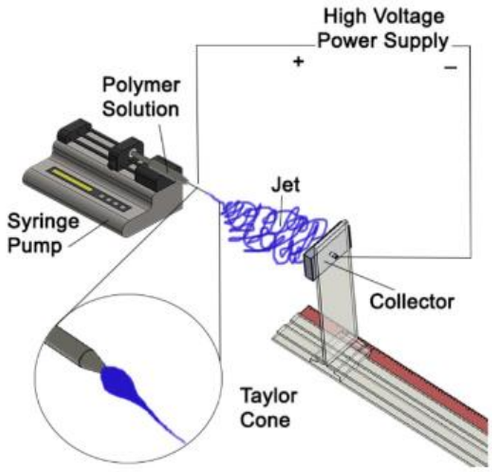

Figure 1 Electrospinning configuration system
Source: (Velasco-Baraza et al., 2016)

In this research work, it is proposed to obtain polymeric nanofibers as a delivery system for Neem (*Azadirachta indica*) extract due to its cytotoxic properties to combat cervical cancer. According to studies by Avalos-Soto *et al*. (2014), ethanolic extracts of *A. indica* showed toxic and cytotoxic activity, being the ethanolic extract of Neem seed the one that presented the highest toxic activity with an LD50 of 476 µg/ml on *A. indica* nauplii, regarding cytotoxic activity the crude extract of Neem leaf was the one that presented the highest activity with an IC50 of 22. 03 µg/ml on the MCF7 line (breast cancer), the hexane fraction of Neem leaf was the most active on the MCF7 cell line (breast cancer) with an IC50 of 25.17 µg/ml; these results allow us to consider it as a potential adjuvant in the treatment of cancer. Therefore, the objective of the present work is to design and automate a horizontal electrospinning system to elaborate a polymeric membrane with nano and microfibers of prolonged release of Neem extract as an alternative for the treatment of cervical cancer.

Materials and Methods

Materials

Poly-ε-caprolactone (PCL) (P.M. 80,000) was purchased from Química, S. de RL. de CV (Mexico). Neem extract was purchased from Extractos Sigma S. A. de C. V. (Mexico). ACS glacial acetic acid and reagent grade acetone were purchased from Fermont® (Mexico). 5 ml sterile syringes with 21G gauge needles and 32 mm length were used. Electronic components from disused computers and printers were recycled for the construction of the electrospinner.

Equipment

UV-Vis spectrophotometer (Thermo Scientific, Genesys 10uv Scanning, USA), inverted platinum optical microscope, (Carl Zeizz, M. AX10Vert.A1, Mexico).

Experimental Methods

Construction of the electrospinning system

Recycled parts were collected from electronic devices: *flayback* (computer monitor), electricity saving light bulb circuit, printer roller, motor and syringes. The parts were assembled on an acrylic platform according to the electrospinning system scheme (Figure 1). Previously the recycled parts such as *flayback*, printer roller and motor were cleaned with alcohol.

Automation of the electrospinning system

The movement of the infusion pump from right to left was automated to avoid agglomeration of the fibers at the time of injection, installing an Arduino board controlled by means of an algorithm to establish the direction and speed of rotation of motors with an L293D driver. The movement of the collector plate was controlled by a 5V motor connected directly to the power outlet with a 5V eliminator. A U.V. lamp was also installed on top of the system for aseptic conditions. The voltage source (*flayback*) was obtained from an old computer monitor, with 10 and 15 Kv characteristics.

Fabrication of polymeric micro and nanofibers

A polymeric solution was prepared employing poly-ε-caprolactone (16.66 % (w/v)) and 50 mg of Neem extract, using acetone as solvent. A blank polymeric solution was prepared at the same concentration, without the addition of extract. The prepared polymeric solution was deposited in a 5 ml syringe coupled with a 21G X 32 mm gauge metal needle. The polymeric solution was injected under continuous flow, using a voltage of 10 kV at a distance of 10 cm from the collector plate, which was covered with aluminum foil where the nanofibers were deposited**.**

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Morphology

Fiber morphology and size were determined by means of an inverted optical microscope (Zeiss), employing a 1000 X magnifying eyepiece. The morphology of poly-ε-caprolactone fibers (PCL-NFs) and poly-ε-caprolactone fibers with Neem extract (PCL-NEEM-NFs) was evaluated.

Encapsulation efficiency of Neem extract

Samples of 50-100 mg of the polymeric membrane were hydrolyzed with 3 ml of [1.3 M] HCl for 48 hours. Subsequently, the samples were neutralized with NaOH [2.5 M]. The systems were filtered with a Millipore 0.22 µm membrane. The filtrate obtained was made up to 10 ml using an 8% (w/v) sodium lauryl sulfate solution (LSS) as solvent (Solution A). Aliquots of 5 ml of Solution A were taken and 20 µl of a 0.5% (w/v) $FeCl_3$ acid solution (Solution B) were added. This solution was heated to a temperature of 60°C for 15 min, allowed to cool to room temperature and its absorbance was determined at a maximum wavelength (λ) of 423 nm.

The amount of loaded extract was obtained from the formula 1:

$$\%E.C.=(CE/CEI)*100 \quad (1)$$

Where E.C., is the amount of extract loaded on the polymeric membrane, C.E, is the amount of loaded extract determined and C.E.I., is the amount of initial extract.

Results and discussion

Construction and automation of the electrospinning system

Figure 3 shows the final prototype of the automated electrospinning system. As a result, a fixed structure was obtained for the injection pump by means of rails, through which the direction and form of injection of the polymeric solutions on the collector plate can be controlled. An Arduino system controlled by a cell phone was coupled to control the injection speed of the infusion pump.

Figure 2 PCL polymeric membranes obtained by the electrospinning technique

Fabrication of polymeric micro and nanofibers

The assembly of the electronic components to assemble the electrospinning prototype was adequate, using such equipment polymeric membranes of poly-ε-caprolactone were obtained. The dimensions of the films formed were 17 x 17 cm in area (Figure 2).

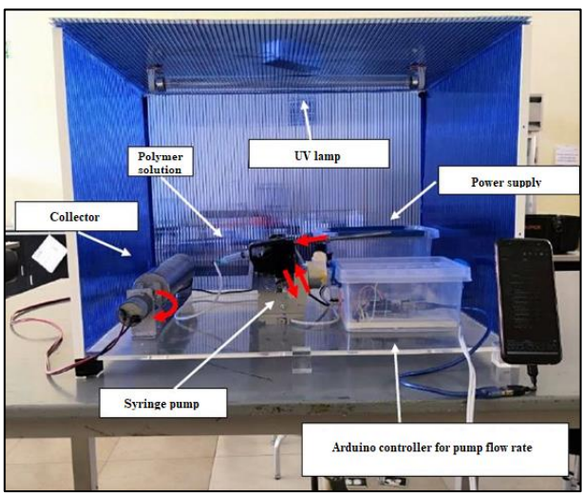

Figure 3 Automated electrospinnng system for obtaining polymeric membranes

Morphology

As shown in Figure 4, the PCL-NFs obtained have a diameter of 28-35 nm. The micrograph of the polymer film indicates a variation between the sizes of the nanofibers, this may be due to the lack of control of the injection rate of the polymer solution, a factor that will be considered for the formation of the following membranes.

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In Figure 5, the polymeric membrane containing Neem extract presents a greater thickness and therefore a larger diameter of the fibers (22-71 nm). This may be due to the presence of the extract, due to an increase in the viscosity of the solution to be injected. However, despite having control over the injection rate, the polymeric membrane still continues to form with differences in nanofiber sizes. This suggests the need to control the injection distance, as well as to control parameters of the polymeric solution, such as: viscosity, type of solvent, concentration, conductivity, among others.

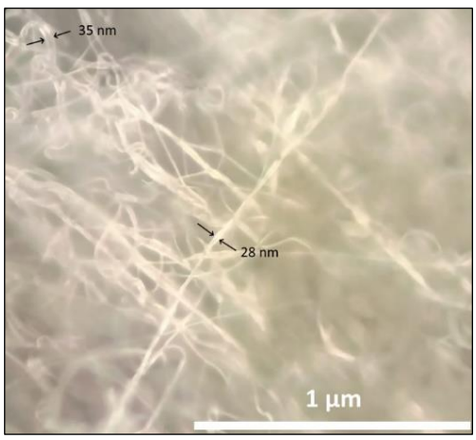


Figure 4 Optical micrograph of the polymeric membrane PCL-NFs. Images taken with a Carl Zeiss inverted optical microscope at 1000x magnification

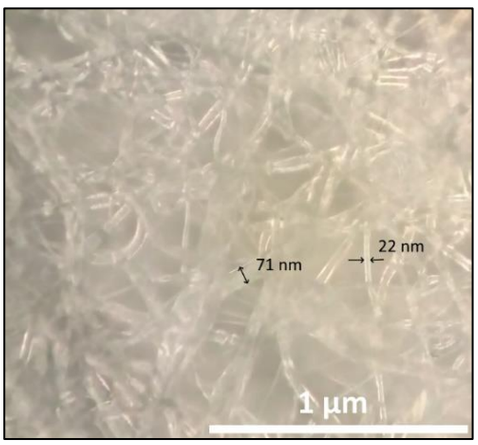
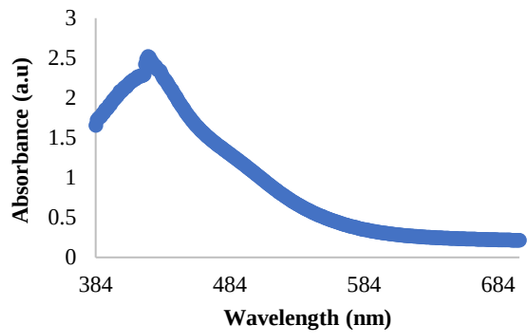


Figure 5 Optical micrograph of the polymeric membrane PCL-NEEM-NFs. Images taken with a Carl Zeiss inverted optical microscope, at 1000x magnification

According to the results, the prototype electrospinning equipment presents some limitations that can be modified and adapted, such as: a) the power supply has a maximum voltage of 15 KV, b) the size of the fibers is dependent on the power of the power supply.

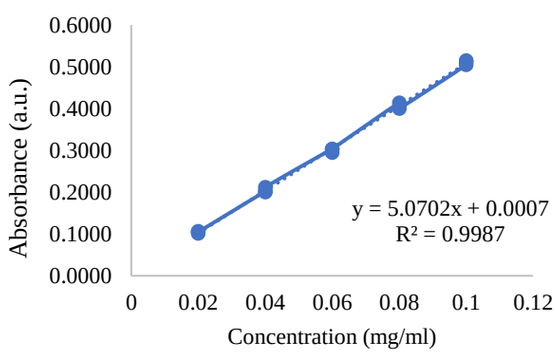
Neem extract encapsulation efficiency

In Graphic 1, the UV-VIS absorption spectrum of the 0.5% Neem extract-FeCl3 colored complex is shown. The absorption maximum occurred at a wavelength of 423 nm. According to Fong Lores et al. (2014), Neem extract, can form colored complex with $FeCl_3$ to determine phenols and tannins, in this study, the colored complex of Neem extract with $FeCl_3$ was formed to quantify its content present in PCL-NEEM-NFs.



Graphic 1 UV-Vis spectrum of the colored complex of Neem-$FeCl_3$ at 0.5%

Graphic 2 shows the linear relationship between the concentration of the colored complex of Neem-$FeCl_3$ extract at 0.5% and its absorbance, in the concentration range of 0.10 to 0.51 mg/ml (r^2=0.99). In Table 1, the results of the calibration curve have a coefficient of variation of 2.24% less than 3%, a criterion established in the Guide for the Validation of Analytical Methods (Garcia et al., 2002), these values indicate that the method is reliable for quantifying the concentration of Neem extract on the polymeric membrane.



Graphic 2 Calibration curve of the 0.5% Neem-$FeCl_3$ colored complex (λ=423nm)

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Concentration (mg/ml)	Absorbance	Abs/conc
0.02	0.1009	5.05
0.02	0.1063	5.32
0.02	0.1048	5.24
0.04	0.2019	5.05
0.04	0.1998	4.99
0.04	0.2115	5.29
0.06	0.3028	5.05
0.06	0.2945	4.91
0.06	0.3026	5.04
0.08	0.4137	5.17
0.08	0.4041	5.05
0.08	0.3997	4.99
0.1	0.5046	5.05
0.1	0.5148	5.15
0.1	0.5115	5.12
	Average	**5.09**
	Stand. Deviation	**0.11**
	%C.V.	**2.25**
Note: C.V. Coefficient of variation		

Table 1 Calibration curve to evaluate the linearity of the UV-VIS spectrophotometric method for the quantification of Neem-$FeCl_3$ at 0.5% (λ=423nm)

The calibration curve allowed us to quantify the amount of Neem extract loaded on the polymeric membrane. The percentage of encapsulation efficiency was 78.4%, a favorable result, however, further studies will be necessary considering a higher load of extract to be loaded on the polymeric nanofibers.

Conclusions

A prototype of electrospinning equipment was built and automated from recycled parts of electronic components. The prototype allowed us to obtain a polymeric membrane of PCL-NEEM- NFs, with diameters in the range of 22-71 nm. The variation in the average diameter of the nanofibers suggests that it is necessary to have greater control over the infusion rate of the polymeric solution to obtain more homogeneous fiber diameters, in addition to the need to correlate the parameters of the polymeric solution to optimize the obtaining of homogeneous membranes. The method to quantify the Neem extract in PCL-NEEM-NFs allowed us to determine that we were able to obtain 78.4% encapsulation efficiency. The PCL-NEEM-NFs film may be a viable alternative for cytotoxicity studies with cervical cancer cell models.

Acknowledgments

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