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Preparation and Characterization of Coaxial Electrospun Fibers Containing Triclosan for Comparative Study of Release Properties with Amoxicillin and Epicatechin

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Abstract: The optimal conditions for the fibers preparation of cellulose acetate (CA) and poly(vinyl pyrrolidone) (PVP) containing triclosan within the fiber were successfully found; the physicochemical characteristics of these fibrous membranes were corroborated by FTIR spectroscopy, thermal analysis, mechanical tests, SEM , and TEM analysis. The formation of composite fibers of CA and PVP containing triclosan at the core of the fiber was evidenced. A comparative study of the release properties of amoxicillin, epicatechin or triclosan embedded into fibers CA/PVP/CA was performed. As more interactions of the drug with CA or PVP occur, slower release of the drug into the release medium takes place. Regarding the drug delivery system design, it is important to consider the possible molecular interactions between the material components and predict how fast or slow the drug will be delivered into the corresponding medium.

Keywords: Amoxicillin, epicatechin, release, system design, triclosan, molecular interactions.

1. INTRODUCTION

Different polymeric materials are used today for the release of drugs, among them are poly (vinylidene fluoride-trifluoroethylene)/NAY zeolite [1], poly (ε-caprolactone) [2], synthetic polymers [3], polymeric micelles [4], biopolymers as cellulose [5], among others. Different methods of preparing materials with drug delivery properties are reported including electrospinning, a versatile and easy method [6-9]. The synthesis and design of materials for safe and effective drug delivery play an important role in modern biomedical science and medicine [10]. In order to have an optimum material for the release of drugs, the application site should be considered, for example, in oral mucosal [11], for dermal and transdermal delivery [12, 13], for diabetic foot ulcer [14], vaginal route [15], in cardiovascular implants [16], among others. Drug solubility must also be considered, in either water or another solvent, depending on its application [17-19]. A very important aspect to consider when designing a material with good release properties of a specific drug, are the interactions with the molecular environment of the material from which it releases [20, 21], as well as the chemical interactions between the drug and the material components.

We have been reporting in our previous papers the preparation, characterization and release of different types of drug embedded inside the fibers made of cellulose acetate (CA) and poly (vinyl pyrrolidone) (PVP) obtained by electrospinning, amoxicillin [22], and epicatechin [23]. Triclosan (Tr) is a widely studied drug that has been used for several years; there are reports of its properties as an antimicrobial agent [24-27], as well as being the cause of problems, like toxicity and adverse effects [28-32]. We used the same system described in reference 22, but in this work, the drug used was Triclosan. Here, we prepared fibers of CA and PVP containing Tr, in order to make a comparative study of the release behavior of amoxicillin, epicatechin and triclosan. The effect of the chemical structure of each drug in the formation of molecular interactions with the other two components of the fiber was studied, correlating this with the results obtained in the drug release kinetics.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study included cellulose acetate (CA) powder, 39.7 wt% acetyl content, with an average Mn of 50,000 (Aldrich); poly(vinylpyrrolidone) (PVP) white

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powder and average Mw of 360,000 (Aldrich); acetone, 99.7% (Aldrich); ethanol, 99.6% (Fagalab); triclosan, (Aldrich). All reagents were used as received.

2.2. Methods

2.2.1. Fibrous Membrane Preparation by Coaxial Electrospinning

For the preparation of a cellulose acetate-poly(vinylpyrrolidone) fibrous membrane, CA/PVP-Tr/CA with triclosan incorporated into the fiber, a careful study of the variables was performed, according to our previous work [22, 23]. The variables studied were the flow of the solution and the applied voltage. The concentration of the polymer solution, and the distance between the needle and the collector plate were constant. In the case of the CA solution, an acetone-water mixture was used as a solvent with a polymer concentration of 8% wt. For 4 mL of a PVP solution an ethanol-water mixture was used with a polymer concentration of 8 % wt. and 0.5 g of triclosan were added to the last solution. The solutions were transferred to the plastic syringes and pumped with a syringe pump (KDS Scientific model 200), whose flow rate varied in the range of 0.3-3.0 ml h^{-1}. A high voltage of 11-18 kV was applied to the polymer solution, using a high-voltage power supply (Spellman, model CZE 1000R). Finally, the distance between the needle and the collector plate was set at 15 cm. A square plate of aluminium (10 cm x 10 cm) was used as a collector. The variables were modified until a fibrous membrane was obtained.

2.2.2. FTIR Characterization

Each component of membrane, CA, PVP and Tr was mixed with KBr powder and pelletized. The IR characterizations were performed using a Nicolete Protégé 460 FTIR spectrometer. The membranes CA/PVP-Tr/CA before and after of Tr release, were characterized using an attenuated total reflectance (ATR) technique with a spectrometer Perkin Elmer Spectrum two mode ATR, in the range of 4000-450 cm^{-1}.

2.2.3. Thermal Analysis

Thermogravimetric analysis (TGA) was carried out, using a Perkin Elmer Thermogravimetric analyzer Pyris 1 TGA. Samples of approximately 4 mg were taken, and heated from room temperature to 600°C, with a heating rate of 10°C min^{-1}, under a continuous flow of air at 23 mL min^{-1}.

2.2.4. Mechanical Analysis

A tensile test was performed in dry state using a micro tensometer equipped with a 250 g load cell at a constant crosshead speed of 1.2 mm min^{-1}. Before and during testing, the samples were conditioned at 60% relative humidity and a room temperature of 25°C.

2.2.5. SEM Imaging

The morphology of the fibrous membrane was evaluated using a JEOL 5410LV scanning electron microscope (SEM), operated at both, 15 kV, and 20kV. The samples were gold-sputtered prior to the SEM examination.

2.2.6. TEM Imaging

For the transmission electron microscopy imaging (TEM), a sample of the membrane was placed on a carbon film supported by a copper grid in order to obtain electron micrographs in a JEOL 1010F Electron Microscope.

2.2.7. Release of Triclosan

To study the release kinetics, the membrane samples (10 cm x 10 cm) containing triclosan were in ethanol, because Tr is poorly soluble in water. Said membrane samples were immersed in 400 mL of ethanol and were maintained with continuous magnetic agitation at 25°C. The drug released into the ethanol was investigated. At certain time intervals, an aliquot of 3 mL was taken from the release system; the cumulative amount of triclosan released at each time sampling was determined by UV-VIS spectroscopy at 282 nm using a Perkin-Elmer Lambda 20 UV-vis spectrophotometer system, the 3 mL sample was returned to the release system after this characterization. This experiment was repeated until the absorbance values were constant, that means, when the equilibrium of the released drug was reached, care was taken that no ethanol evaporated from the system. The concentration of released triclosan was determined by interpolation of absorbance values in a calibration curve previously developed.

3. RESULTS AND DISCUSSION

After a systematic study varying the mentioned conditions above, for the coaxial electrospinning method, the optimal conditions for the preparation of cellulose acetate-poly(vinyl pyrrolidone) fibers with embedded triclosan (CA/PVP-Tr/CA) were found; flow rate of 2.2 ml h^{-1} and a high voltage of 15 kV were applied to the polymer solution.

Fig. (**1**) shows the FTIR of CA (a), PVP (b) and Tr (c). The following characteristic peaks were observed: for CA there is a broad band at around 3575 cm^{-1}correspond to the O-H stretching vibrations, C-H stretching vibrations of methylene between 2800-3000 cm^{-1}, an C=O ester stretching peak at around 1759 cm^{-1}, Fig. **1**(**a**); for PVP, a broad band at around 3452 cm^{-1}correspond to the O-H stretching vibrations, due to adsorbed water [33].

The band at 2934 cm^{-1} corresponds to stretching vibrations -CH_2-, the band at 1653 cm^{-1} is attributed to the C=O stretching vibration of the amide group [34], Fig. **1**(**b**); for triclosan, the existence of aromatic rings is normally determined at 3309 cm^{-1} from the CH and C=C ring-related vibrations O-H group [35], C-Cl stretching peaks were observed at 800-600 cm^{-1}, Fig. **1**(**c**).

The ATR-FTIR spectroscopy of the fibers was performed in order to corroborate the existence of triclosan in CA/PVP-Tr/CA before and after the drug release. For the composite material CA/PVP-Tr/CA, the characteristic peaks of each component, CA, PVP and triclosan, were observed, demonstrating the existence of triclosan in the fiber, Fig. **2**(**a**). After the drug release, process the peaks of PVP and triclosan almost disappeared, demonstrating the lack of PVP and triclosan from the fiber, Fig. **2**(**b**); to further analyze this, the spectral region from 4000 to 2000 cm^{-1} is presented in Figs. **2**(**c**) and **2**(**d**), showing the characteristic peaks of each component.

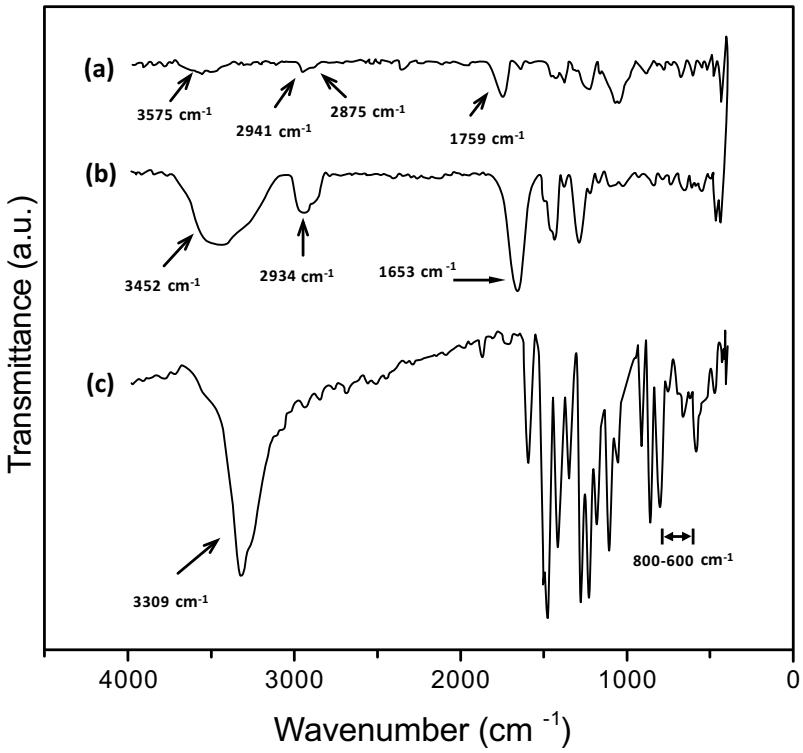

Fig. (1). FTIR spectra of (**a**) CA, (**b**) PVP, (**c**) triclosan.

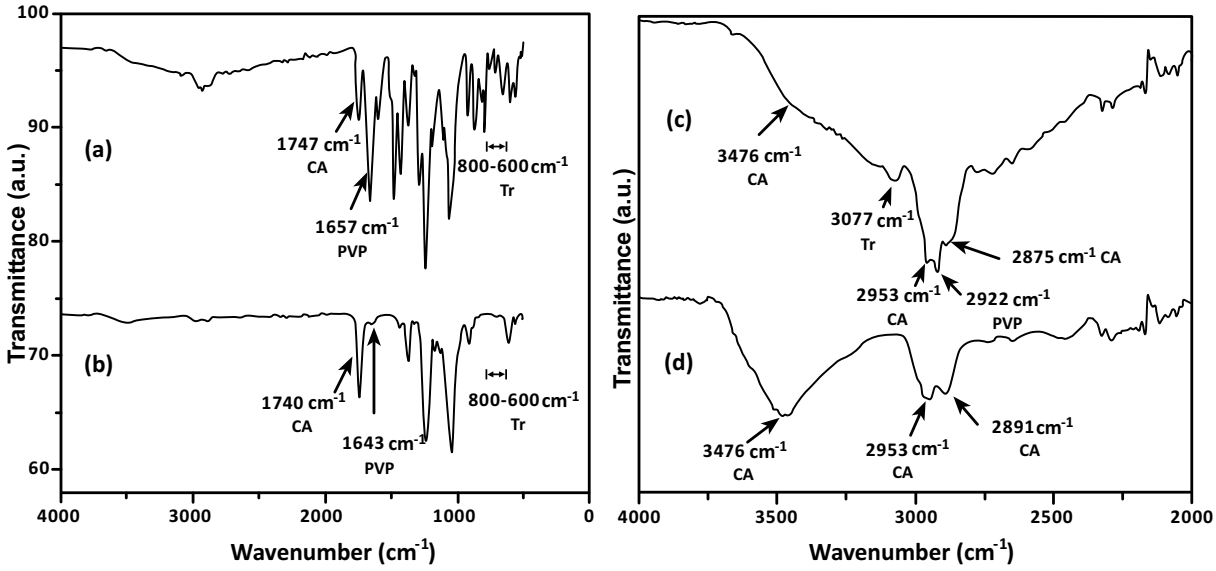

Fig. (2). ATR-FTIR spectra of (**a**, **c**) CA/PVP-Tr/CA before, and (**b**, **d**) CA/PVP-Tr/CA after drug release into ethanol.

In order to observe some kind of interaction, possibly hydrogen bonds, between the components of the material, a comparison of certain characteristic peaks of each component in their pure state (Fig. **1**), and in the composite spectrum (Fig. **2**) is presented.

Shifts in the signals of each component can be observed: (1) CA: ester C = O stretching peak (2) PVP: the band at 1653 cm^{-1}, attributed to the C = O stretching vibration of the amide group, and (3) Tr: vibrations of OH group, when it is part of the composite.

These results are consistent with the results reported by several authors [36-39]. As it is known, for the formation of a hydrogen bond, a hydrogen donor molecule and an acceptor are needed. In this system, CA/PVP-drug/CA, apparently hydrogen bonds are formed between each of the drugs with the other two components of the membrane, CA and PVP. According to the established definition of hydrogen bonding [42, 43], we suggest that the release properties of the three drugs studied here, are governed by the possible formation of conventional and strong hydrogen bonds type O - H---O = C, between the composite components.

Analysis of weight loss as a function of temperature was performed for the CA/PVP-Tr/CA membranes. Fig. **3**(**a**) shows the thermogram of each component of these membranes. Fig. **3**(**b**) shows three steps of thermal degradation between 150°C and 500°C, marked as (1), (2) and (3), these are attributed to triclosan, CA and PVP, respectively; this assumption was made based on the analysis of weight loss of CA, PVP and triclosan separately. Fig. **3**(**c**) shows the thermogram of CA/PVP-Tr/CA after the release process, and it can be observed that the weight loss assigned to PVP and triclosan disappear, and only one weight loss, attributed to CA, appears. It is common to use weight loss analysis to demonstrate the composition of a composite material and the loss of its components in function of temperature [40,41], in this case the drug release. These results are consistent with those obtained in FTIR and ATR-FTIR studies of the membrane after the triclosan release.

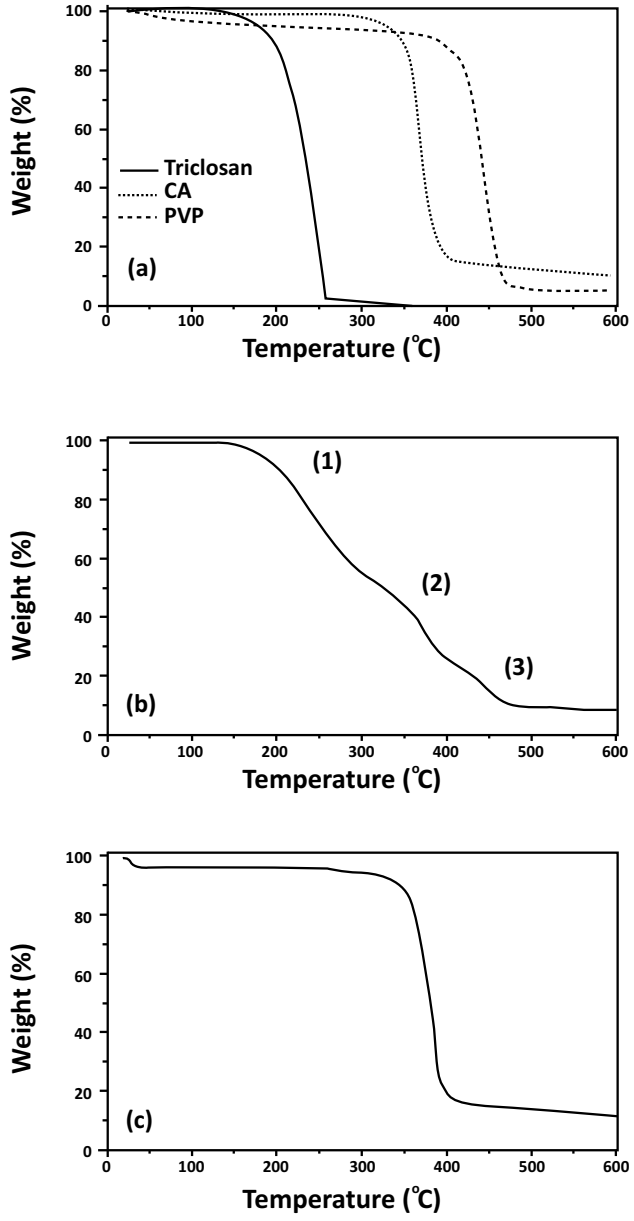

Fig. (3). Thermogram of (**a**) CA, PVP, and triclosan, (**b**) CA/PVP-Tr/CA membrane, (**c**) CA/PVP-Tr/CA after their release into ethanol.

The presence of triclosan on the CA/PVP/CA fibers seems to have an effect on its mechanical properties. With the inclusion of triclosan, the elastic modulus decreases notoriously (30% approximately). The tensile strength is also affected by the triclosan itself, because this value decreases by approximately 41%. The elongation at break has a similar behavior as the tensile strength, because it decreases by more than 50%. These results are shown in Table **1** and indicate the existence of some interaction of triclosan with other membrane components.

Table 1. Values of Elastic Modulus, Tensile strength, and Elongation at break of CA/PVP/CA and CA/PVP-Tr/CA.

Material	Elastic Modulus (MPa)	Tensile strength (MPa)	Elongation at break (%)
CA/PVP/CA	35.88 ± 5.25	3.55 ± 0.58	28.63 ± 4.72
AC/PVP-Tr/AC	24.34 ± 1.83	2.01 ± 0.05	13.62 ± 2.57

To study the morphology of the membranes and verify that the fibers were formed, SEM micrographs were obtained. Fig. **4**(**a**) shows the fibrous membrane CA/PVP-Tr/CA before the release of triclosan. Fibers with cylindrical shapes and diameter values around 1.0 μm can be observed. In the case of the CA/PVP-Tr/CA membranes after the drug release, deformed fibers with clear deterioration can be observed without the original cylindrical shape, Fig. **4**(**b**), suggesting that PVP and triclosan were removed from the fiber.

Fig. **5**(**a**) shows the TEM micrographs for the fibrous membrane CA/PVP-Tr/CA before the release of triclosan, homogeneous fibers with a dark area at the core of the fiber can be seen, there are no empty spaces compared to Fig. **5**(**b**), after the release of triclosan and PVP.

The triclosan released kinetics from a CA/PVP-Tr/CA membrane showed a final triclosan release maximum percentage of 85 % ± 3 in a time of 170 min in ethanol, (Fig. **6**). The release of triclosan in ethanol was studied until equilibrium was reached.

The effect of the chemical structure of the drug embedded in the fiber CA/PVP/CA was analyzed through the kinetics of release. Fig. (**7**) shows the chemical structures of (a) amoxicillin, (b) epicatechin, and (c) triclosan; which were developed using the program Chem draw.

A comparative study of the release properties of the three different drugs embedded in the fibers CA/ PVP/CA was performed. Considering the chemical structures of the three cases studied in this paper, amoxicillin [22], epicatechin [23] and triclosan; amoxicillin has a greater number of functional groups (7) has the ability to interact, and establish hydrogen bonds, with the other components of the fibers, CA and PVP. Following amoxicillin, epicatechin has 5 possible molecular interaction sites, and finally, triclosan has fewer potential interaction sites (1). These possible sites of interaction are marked in (Fig. **7**) as*.

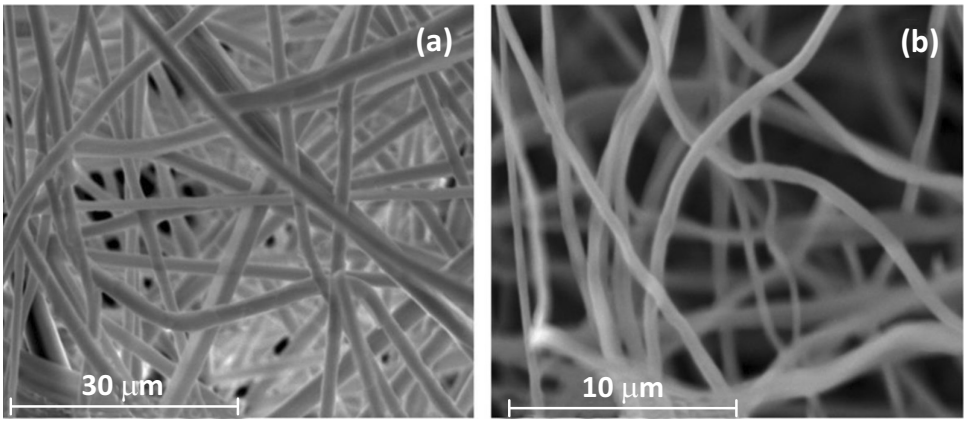

Fig. (4). SEM micrographs, (**a**) for the CA/PVP-Tr/CA membrane before triclosan release, (**b**) after triclosan release into ethanol, at (**a**) 2000x and (**b**) 5000x magnification.

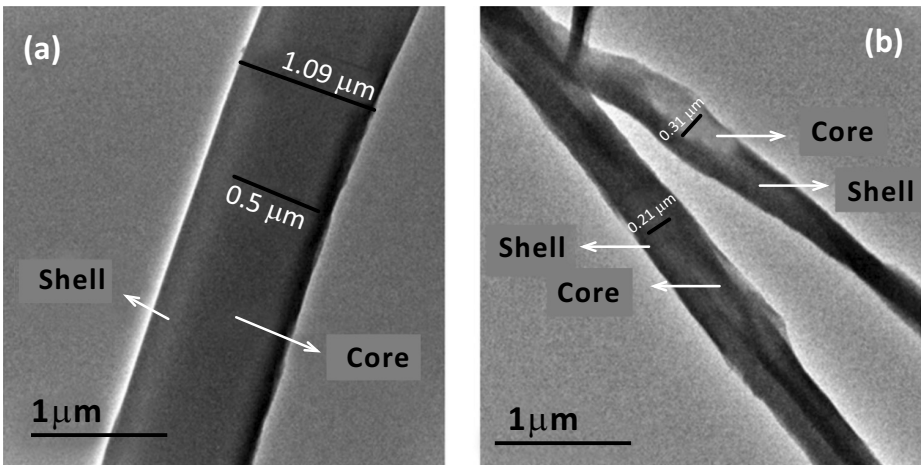

Fig. (5). TEM micrographs for the fibrous membrane CA/PVP-Ts/CA (**a**) before triclosan release and (**b**) after triclosan release into ethanol, at (**a**) and (**b**) 5000x magnification.

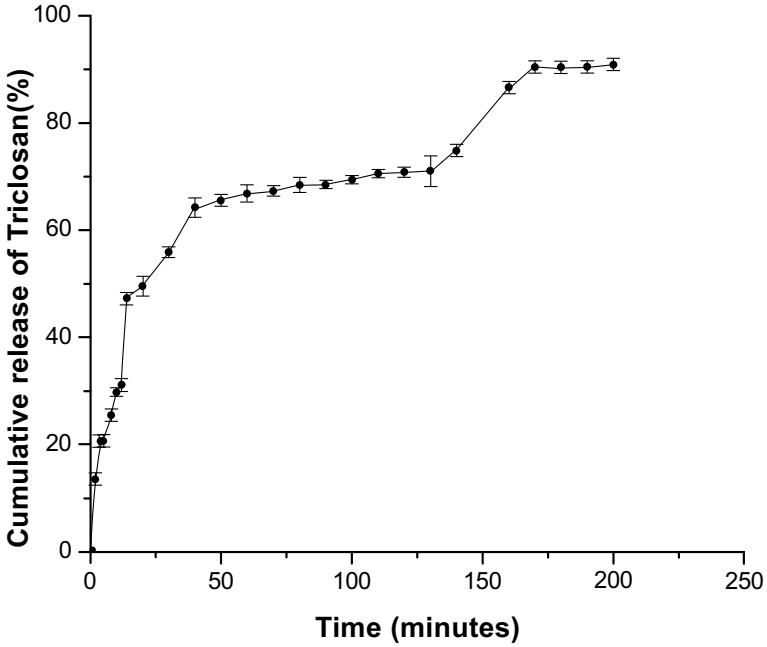

Fig. (6). Kinetics of release of triclosan from CA/PVP-Tr/CA membrane in ethanol.

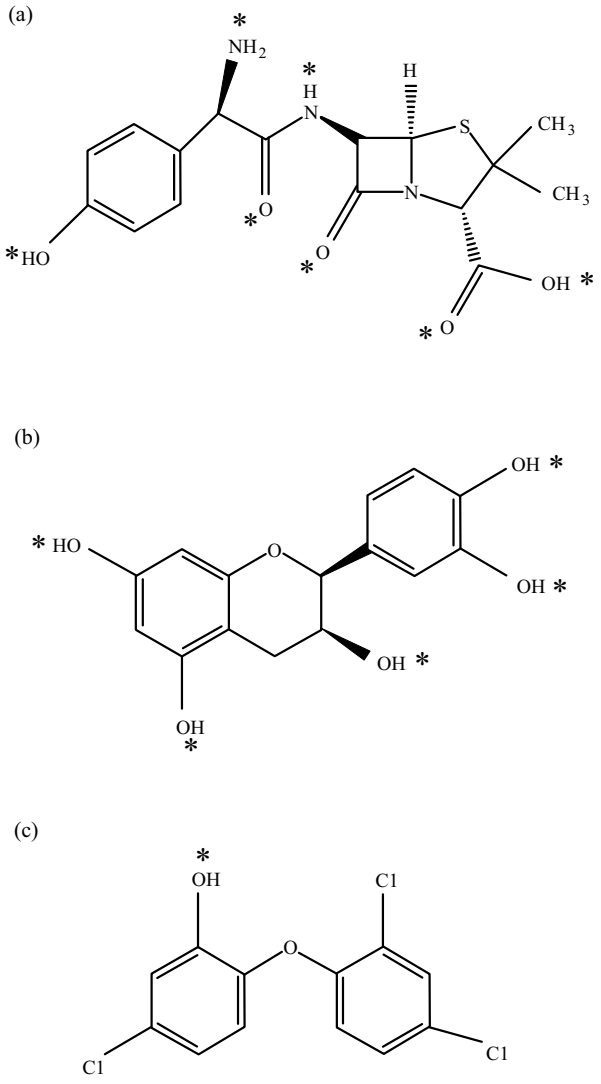

Fig. (7). Chemical structure of (**a**) amoxicillin, (**b**) epicatechin, and (**c**) triclosan.

The cumulative release in equilibrium of the different drugs were: amoxicillin 79.0% at 2880 minutes [22], epicatechin 79.6% at 240 minutes [23], and triclosan 90.4% at 170 minutes, in accordance with the number of sites with the possibility of a molecular interaction with the other two components of the fiber in each drug. A small number of interaction sites of the drug, with the components of the fiber, results in a higher percentage of drug released. Table **2** summarizes the results thus obtained.

In general, considering the time to reach equilibrium, this increased as the number of interaction sites in the chemical structure of the drug increased. These results can be explained by the formation or no formation of hydrogen bonds. Then, according to the results obtained, it is proposed that for the release of drugs from the fiber core to the surrounding medium, the molecular structure of drug release that takes place is in greater or lesser degree depends on the number of possible interactions between the drug and the other two components of the fiber. More interactions between the drug and the two polymers that form the fiber, CA or PVP, will conduct a slower release of the drug into the release medium.

Table 2. Release properties for electrospun CA/PVP-drug/CA membrane.

Drug	Number of potential sites hydrogen bonding	Drug release at equilibrium (%)	Time to reach equilibrium (min)
Amoxicillin [22]	7	79.0	2880
Epicatechin [23]	5	79.6	240
Triclosan	1	85.0	170

CONCLUSION

The optimal conditions for the preparation of fibers of cellulose acetate and poly(vinyl pyrrolidone) containing triclosan within the fiber were found; the physical, chemical, and morphological characteristics of these fibrous membranes were corroborated by FTIR and ATR-FTIR spectroscopy, thermal analysis, mechanical tests, SEM, and TEM. The formation of composite fibers of CA and PVP containing triclosan in the core of the fiber was evidenced. A comparative study of the release properties of different drugs embedded in the fibers CA/PVP/CA was performed. As more interactions of drugs with CA or PVP, less of the drug was released into the medium. For the design of drug delivery systems, it is important to consider the possible molecular interactions between the material components in order to predict how quick or prolonged the drug delivery it will take in the medium in which it will be applied.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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PATIENT CONSENT

Declared none.

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