

Article

Bacterial Cellulose–Polyvinyl Alcohol Based Complex Composites for Controlled Drug Release

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Featured Application: The developed matrices could be used as sustainable drug delivery systems (transdermal patches). Mathematical modeling provides numerical evaluation of the phenomena occurring during drug release and valuable predictions on release behavior overall.

Abstract: Drug-loaded mono- and multilayer composite membranes were prepared. The composites, based on nano-fibrillated bacterial cellulose, nano-powdered bacterial cellulose, and polyvinyl alcohol, all biocompatible and biodegradable, were characterized in terms of basic factors related to drug diffusivity and mass transfer: swelling ability, water solubility, and water vapor permeability. Tetracycline hydrochloride was used in this case as drug model. Drug release was evaluated in an aqueous environment for two concentration levels of the antibiotic, and mathematical modeling was applied to fit experimental data. Tetracycline release was influenced by a membranes' structure, layers' composition, and by a membranes' thickness. Bacterial cellulose nanofibrils proved to be the key factor in achieving suitable drug release profiles. Thus, sustained antibiotic delivery was obtained for several days in the case of multilayer composites. The composites proved drug stability and antibacterial efficiency before and after TC-HCl continuous release for several days.

Keywords: polyvinyl alcohol; bacterial cellulose; multilayer composites; diffusion; controlled release; mathematical modeling



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1. Introduction

Drug delivery systems, in general, have been developed and commercialized for decades, but at the same time, they are still extensively researched. In particular, there is a constant search for new biocompatible nanostructured systems to be used as effective anti-infection wound dressings [1,2]. Traditionally, wound dressings (WD) were designed to absorb exudate, to keep the wound area moist, and to allow gas (oxygen) transfer. Nowadays, they are designed not only to control moisture, but also to provide flexibility, softness, no cytotoxicity, transportation, and sustainable release of active substances [3]. Considering antibacterial activity, improved WD should prevent drug inactivation or degradation and allow for the maintenance of an optimal drug concentration for prolonged periods of time, lowering toxic or harmful side effects, and minimizing drug levels variations [4,5]. Most recently, researchers found that multilayer composites could be a better alternative to monolayer WD to meet as many requirements as possible [6].

This work aimed to develop a monolayer and multilayer (three layer) anti-infection WD, using a specific combination of polyvinyl alcohol (PVA), nano-powdered bacterial cellulose (NpBC), and nano-fibrillated bacterial cellulose (NfBC). Obtained composites were loaded with tetracycline hydrochloride (TC-HCl). The choice of TC-HCl as the model drug was made due to its broad-spectrum activity as an antibacterial agent [7]. Furthermore TC-HCl is one of the most used antibiotics in medicine (human and also veterinary).

Cellulose (from plants of bacterial origin) and PVA, well known as biodegradable and biocompatible polymers, are much used in the medical field for numerous applications [8,9], including as single layer or multilayer WD [5,10].

BC, as versatile biopolymer, with exceptional conformation and properties, is extensively studied and used in a wide variety of applications/industries, as many review articles are indicating, including very recent ones [11–18]. BC favors cell adhesion and proliferation; its deformability resembles soft tissue and is non-allergenic. Furthermore, its degradation product is glucose; thus, the usage of BC as biomaterial no longer needs to be questioned [19]. Biocompatibility, non-toxicity, and high liquid retention capacity are the main features that recommend it for drug delivery and wound healing [14]. In this work, two forms of BC were used to investigate the effect on drug release, since it has been proved that by disrupting the natural 3D structure of BC (to obtain nanofibrils or nanoparticles) the properties of the resulting composite materials containing them, can be changed (swelling, crystallinity) [20,21].

PVA-based materials are extensively applied in the medical field. Among many attributes (thermal and chemical stability, film forming ability), PVA is well known as non-toxic and biocompatible with living cells/tissues [22]. Moreover, it was shown in previous research that BC improves cells viability in cytotoxicity evaluations of pure PVA [23].

The goal of our research was to develop novel and versatile structures, based on well-known biodegradable and biocompatible materials, to hinder initial burst release and, at the same time, to provide retarded and/or adjustable drug release. Although there are many known preparation methods for wound dressing films, we chose to prepare the composites using the conventional solvent casting method, due to facile and low-cost processing, and suitability for polymeric dispersions [24].

To the authors' knowledge, this is the first study to develop complex composites, based on two forms of BC, to the benefit of different physico-chemical characteristics depending on the BC type, and PVA. Given recent studies reported in the literature, and based on the authors' knowledge and expertise, the resulting composites should exhibit non-toxicity and excellent biocompatibility [23,25].

The main objectives were to evaluate the materials' structural characteristics, water and water vapor transport properties, antibacterial activity and, more importantly, to prove the purposed applicability: controlled drug release. To evaluate TC-HCl diffusivity, the process was assessed experimentally and by mathematical modeling. Mathematical models were applied to fit the experimental data, to evaluate drug release processes, and to identify the influencing factors on the overall mass transfer rate. The obtained materials provided mass transfer limitations necessary to achieve beneficial drug release.

2. Materials and Methods

2.1. Materials

BC was obtained by us in the Mass Transfer Laboratory of University Politehnica of Bucharest, following previously described procedures [26]. Briefly: BC membranes were produced in 2 L rotating biofilm contactors on Hestrin–Schramm medium in ambient conditions (25 °C, atmospheric pressure) and 60 rpm for 10–12 days. The obtained gel-like pellicles were purified by boiling in NaOH 0.1N. To remove impurities and microbial cells, the pellicles were thoroughly washed with deionized water until obtaining a neutral pH. BC wet membranes were grounded with a colloidal mill to obtain NpBC and a knife mill to obtain NfBC.

To obtain composite membranes, TC-HCl powder, BioReagent, and PVA average molecular weight $M_w = 145,000$ g/mol, 99% hydrolyzed purchased from Sigma–Aldrich Chemie GmbH, Germany, were used.

2.2. Composites Preparation

To prepare monolayer composite samples, NpBC was dispersed under continuous stirring in varying proportions in a 7% (*w/w*) PVA aqueous solution. This solution was obtained by dissolving PVA flakes in water at 90 °C. Active membranes containing the antibiotic were prepared with the addition of TC-HCl at different amounts, directly in 100 g PVA-NpBC suspension (0.1 g, respectively, 0.5 g/100 g resulting solution). The mixture

was then homogenized using a magnetic stirrer for 1 h at room temperature. Samples were further deaerated for approximately 3 h using a vacuum system. The resulting mixtures were poured in plastic Petri dishes and allowed to dry at room temperature until they reached a constant mass (approximately 24 h). After drying, the membranes were removed from the cast plates and their thickness was measured to the nearest 0.001 mm by a micrometer (Mitutoyo Mfg Co. Ltd., Japan). The average values of measurements at five random positions were considered, as the films' thickness is necessary to calculate the diffusion properties of membranes. The values together with samples composition and blending ratio of the constituents are presented in Table 1.

Table 1. Structure, composition, and thickness of composite membranes.

Sample ID	Structure	Composition	Blending Ratio (w/w) *	Thickness (mm)	Drug Loading mg/g d.m.
M0	monolayer (control)	PVA, NpBC	7, 2.3	0.479 ± 0.395	NA
M1	monolayer	PVA, NpBC, TC-HCl	7, 2.3, 0.1	0.481 ± 0.121	10.60
M2	monolayer	PVA, NpBC, TC-HCl	7, 2.3, 0.5	0.523 ± 0.158	50.86
M1p	3-layer	PVA/M1/PVA	7/7, 2.3, 0.1/7	0.756 ± 0.142	7.73
M2p	3-layer	PVA/M2/PVA	7/7, 2.3, 0.5/7	0.785 ± 0.155	35.71
M1pf	3-layer	PVA, NfBC/M1/PVA, NfBC	7, 1.75/7, 2.3, 0.1/7, 1.75	0.793 ± 0.151	7.24
M2pf	3-layer	PVA, NfBC/M2/PVA, NfBC	7, 1.75/7, 2.3, 0.5/7, 1.75	0.817 ± 0.148	35.20

* Weight in g of each constituent per 100 g casting solution.

Multilayer systems were accomplished by coating previously obtained active membranes, designated as the inner layer, with two external layers of a specific composition, as will be further detailed. The external layers were obtained by pouring 25 g of: (a) simple PVA 7% (w/w) solution, and (b) a mixture achieved by blending PVA 7% aq. solution with NfBC to a PVA:NfBC 4:1 ratio on each side of the active membrane (inner layer). When one side was coated with the external layer solution, it was allowed to dry at 80 °C for 3 h, and then the other side was also coated.

Based on used solutions' volumes and constituents' concentrations, the drug loading of the resulting membranes was determined and presented in Table 1.

2.3. Composites Characterization

The microstructure and the morphology of the materials was evaluated using a FEI Quanta Inspect F electron microscope (FEI Company, Hillsboro, OR, USA), operated at 20 kV. A thin and continuous layer of gold was deposited on the samples' surface before SEM investigation.

The behavior of obtained systems in an aqueous environment was studied. Two parameters were determined: swelling degree, as a measure of moisture uptake capacity, and water solubility, as a parameter indicating matrix degradation and biodegradability.

To determine the *swelling degree (SD)*, membranes were dried to a constant weight. Then, each sample was prepared by cutting it into 2 × 2 cm square shapes and immersed in distilled water at room temperature (25 °C) for 2 h. The experiments were performed in triplicates. Based on the authors' best knowledge and a survey of the literature, 2 h were considered sufficient for equilibrium achievement [27–29]. With a very short time needed for the procedure, the membrane dissolution into the distilled water was neglected. Furthermore, the amount of TC-HCl contained in the active films and released in aqueous media was considered insignificant compared to the amount of absorbed water. The weight of each swollen sample was measured after gently blotting the wet surface with tissue paper to remove excess water.

Swelling degree (SD) was obtained by measuring the initial weight, m_i (g), and the weight of the sample in a swollen state, m_s (g) using Equation (1):

$$SD = (m_s - m_i) / m_i \quad (1)$$

Water solubility (WS) was also determined in triplicate, in accordance with the method proposed by Shen et al., 2010, but slightly modified [30]. Samples of membranes (square shapes of 2 × 2 cm) were dried to a constant weight, and then immersed in glass beakers

containing 50 mL distilled water. The beakers were periodically shaken and kept at room temperature (25 °C) for a longer time (72 h). Undissolved pieces of membranes were removed from the water and further dried to a constant weight. The water solubility of films was calculated according to Equation (2), where m_i is the initial weight of the sample, and m_f is the weight of the dried un-dissolved sample, both measured in grams:

$$WS = (m_i - m_f) / m_i \quad (2)$$

Water vapor permeability (WVP) of each composite (prepared as monolayer or multi-layer) was determined in triplicate. We followed a well-known procedure, earlier described by Limpan et al. [31]. The composites were cut into disks with a diameter of 3 cm. Each disk was carefully sealed on a cup containing silica gel (0% RH) and placed in a desiccator equipped with a sensor for temperature and relative humidity. The desiccator contained distilled water as moisture source. The experimental conditions were 37 °C and 95% RH, maintained constant for the entire experiment. The absorbed moisture was determined periodically by the weighing of the cups. The slope of variation in weight vs. time (after approximately 8 h, when steady state was reached) was obtained by linear regression. Correlation coefficients for all samples were higher than 0.999. The water vapor transmission rate (WVTR) was calculated by dividing the slope by the film area. WVP was determined according to the following equation [32]:

$$WVP = WVTR \cdot \delta / \Delta p \quad (3)$$

where: *WVP* is water vapor permeability ($\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$), *WVTR* represents water vapor transmission rate through film ($\text{g m}^{-2} \text{s}^{-1}$), δ is the thickness of the film (m), and Δp represents water vapor partial pressure difference across the two sides of the coatings (Pa).

2.4. Drug Release Studies

Aiming to observe the influence of structural configuration and layers composition, the investigation of drug release was conducted in a simple system: 100 mL water was used as a desorption solution, at a constant temperature (25 °C), under continuous stirring to accelerate diffusion and maintain a homogenous diffusion environment. The experiments were performed in an apparatus similar to the one reported by Guiga et al., 2010 [33]. Samples of the solution (0.1 mL) were removed at predefined time intervals and the concentration of TC-HCL was measured by a UV/VIS spectrophotometer (CINTRA 6-GBS Scientific-Australia) at 365 nm. The samples' volume was considered very low to influence diffusion.

Antibiotic release was modeled using the classical unsteady state Fick's diffusion equation. Films were considered as plane thin sheets with an initially homogenous TC-HCL concentration distribution and constant diffusivities (in a simplistic approach):

$$\frac{\partial C}{\partial \tau} = D(\tau) \cdot \frac{\partial^2 C}{\partial x^2} \quad (4)$$

where: *C* is the drug concentration (kg m^{-3}), *D* is the diffusion coefficient ($\text{m}^2 \text{s}^{-1}$), *x* is the space coordinate in the diffusion direction (m), and τ is the immersion time (s).

When the stirring rate of the release medium provides the perfect mixing conditions, the amount of drug released after a period of time can be calculated with Equation (5) [34]:

$$M_{\text{Fickian}}(\tau) = M_{\infty} \cdot \left(1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \cdot \exp \left(-\frac{(2n+1)^2 \pi^2}{4\delta^2} \cdot D \cdot \tau \right) \right) \quad (5)$$

where: M_{∞} is the amount of TC-HCL released at equilibrium (kg) and δ is the film thickness (m).

Overall diffusion coefficients were calculated using Equation (6), valid for the early stages of diffusion:

$$D = \left(\frac{k' \cdot \delta}{4} \right)^2 \pi \quad (6)$$

where: k' is the slope of the linear regression of M_t/M_∞ , versus $\tau^{1/2}$.

Additionally, the two-stages washing/diffusion mathematical model of So and MacDonald (1986) was used to describe TC-HCl release from multi-layered membranes. The following exponential equation was adapted for this study [35]:

$$c^* = c_w^* \cdot (1 - e^{-k_w \cdot t}) + c_d^* \cdot (1 - e^{-k_d \cdot t}) \quad (7)$$

where: $c^* = c/c_\infty$ is the solute concentration in the solution (solvent) at any time of the diffusion/release process, mg L^{-1} ; c_∞ is the hypothetical TC-HCl concentration in the solution at equilibrium (at time equal to infinity), mg L^{-1} ; $c_w^* = c_w/c_\infty$, where c_w is the final hypothetical concentration in the solution due to the washing stage alone, after the release process has been completed, mg L^{-1} ; $c_d^* = c_d/c_\infty$, where c_d is the final hypothetical concentration in the solution due to the diffusion stage alone, after the release process has been completed, mg L^{-1} ; k_w is the kinetics coefficient for the washing stage, min^{-1} ; and k_d is the kinetics coefficient for the diffusion stage, min^{-1} .

2.5. Antibacterial Activity

The antibacterial activity was determined using the modified disk diffusion method in solidified culture media [36]. The microorganisms targeted in this study were *Escherichia coli* strain DH5K (Gram-negative bacteria) and *Bacillus subtilis spizizenii* Nakamura, strain ATCC 6633 (Gram-positive bacteria), both from the Industrial Microbiology Laboratory, Department of Chemical and Biochemical Engineering from University Politehnica of Bucharest. The culture media used were Luria-Bertani Agar-LBA (purchased Roth, 2021) for *E. coli*, and nutrient agar (purchased Roth, 2021) for *B. subtilis*. The pH of the culture media was adjusted to neutral (7.4) at 25 °C with NaOH 0.1N. The medium was sterilized in an autoclave at 121 °C for 20 min. Glucose was added sterilely by filtration for LBA. The plates were inoculated by flooding with 1000 microliters of bacterial inoculum (optical density at 620 nm) of 0.8 for *E. coli*, and 0.85 for *B. subtilis*, the excess being removed, and the plates were left in an incubator with controlled humidity for uniform inoculum growth. The composite films samples were cut to a diameter of 6 mm and UV sterilized for 30 min on each side. The experiments were made in triplicates.

3. Results and Discussions

The obtained results and the most important findings will be presented as follows. Multilayer systems performances were studied in comparison with monolayer membranes.

SEM images of the developed materials are presented in Figure 1. There are some important observations to be mentioned, in correlation with selected images. Figure 1a–c showing cross-sectional morphologies of M0, M1, and M2, reveal the influence of antibiotic agent addition. It can be clearly seen that by increasing the TC-HCl concentration, the microporosity of the composite structure increases. Thus, while M0 (control sample with no antibiotic content) presents a denser structure, M2, with the highest TC-HCl content, shows a highly porous 3D structure with interconnected micropores, which is a beneficial property for wound dressing, as a more porous structure will absorb a higher quantity of exudate. Similar observations were reported for Gelatin/Sodium Alginate composites when TC-HCl was added [37]. Figure 1d–f represent images of different surfaces: no cracks or pores can be seen, indicated a good compatibility of composites' components [23]. Pure PVA coating of M2p (Figure 1e), similar to M1p (not presented here), show a uniform and smooth surface, while NfBC can be spotted on the surface of M2f (Figure 1f). Figure 1g–i present, at lower magnification, cross sections of three-layer films, to give an insight on each layer thickness and microporosity (for instance no porosity in the case of the pure PVA

layer—Figure 1i). The obtained SEM images indicate that inner composite layers have pores ranging from 1 to 5 μm diameter for lower TC-HCl content, and up to 10 μm diameter for higher drug content.

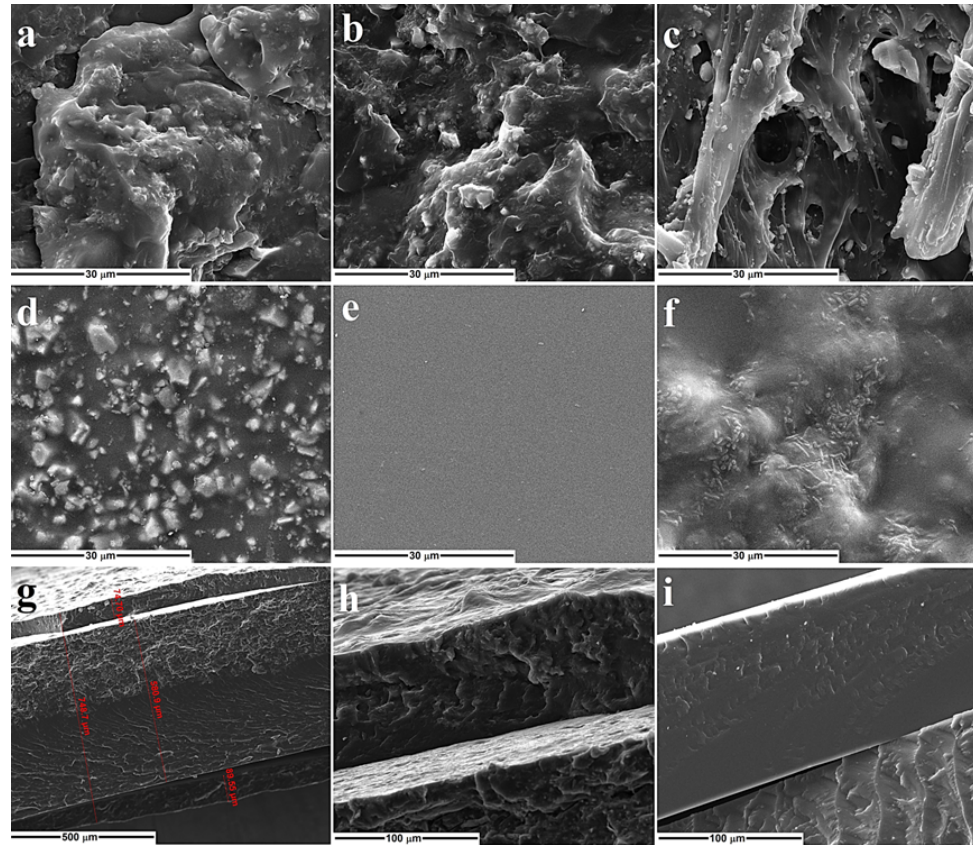


Figure 1. SEM images of the obtained samples: (a) cross section of control monolayer, M0; (b) cross section of M1; (c) cross section of M2; (d) surface of M2; (e) surface of M2p; (f) surface of M2f; (g) cross section of M1pf; (h) detail of M1pf cross section; (i) detail of M1p cross section.

The swelling degree of the obtained membranes is presented in Table 2. The values show high and very high water retention capacities, which is a desired quality for potential wound dressing materials as they are necessary to absorb exudate. It is also well known that the swelling property of a material is directly related to the release of water-soluble drugs [38]. The process of swelling was very fast, and the behavior was expected, considering the composition of the obtained materials. Both BC and PVA have a high hydrophilic nature with high swelling capacity. For instance, Tamahkar reported swelling equilibrium for BC/PVA hydrogels obtained in 45 min, and an SD of maximum 240% [5]. The SD decreases with the increase of BC content as NpBC affects the polarity, crystallinity, and the hydrophilic behavior of PVA. TC-HCl content influenced the SD also. The slight decrease of SD with TC-HCl loading, related to very low drug concentration, may be considered a consequence of the electrostatic attraction between negatively charged BC and positively charged TC-HCl [7]. Overall, in this study, multilayer membranes exhibited higher SD values compared to monolayer membranes.

Table 2. Swelling degree, water solubility, water vapor transmission rate, water vapor permeability of composite membranes, TC-HCl diffusion coefficients, and So and Macdonald model parameters (c_w , c_d , k_w , k_d).

Sample ID	SD (%)	WS (%)	WVTR ($\text{g m}^{-2} \text{ day}^{-1}$)	WVP·10 ⁹ ($\text{g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$)	D ($\text{m}^2 \text{ s}^{-1}$)	R ²	c_w (mg L^{-1})	c_d (mg L^{-1})	k_w (min^{-1})	k_d (min^{-1})	R ²
M1	4.06 ± 0.15	29.80 ± 1.32	271.21 ± 5.63	0.65 ± 0.03	1.045·10 ^{−13}	0.965	4.5	5.81	9.5·10 ^{−4}	5.8·10 ^{−5}	0.989
M2	3.92 ± 0.31	32.44 ± 2.14	255.16 ± 7.59	0.61 ± 0.06	1.029·10 ^{−13}	0.992	10.5	12.6	6.5·10 ^{−4}	5.5·10 ^{−5}	0.985
M1p	5.65 ± 0.41	36.15 ± 2.32	420.05 ± 9.76	1.59 ± 0.14	1.357·10 ^{−13}	0.930	1.7	3.05	8.5·10 ^{−5}	7.2·10 ^{−6}	0.988
M2p	5.42 ± 0.35	38.69 ± 1.59	387.31 ± 4.95	1.52 ± 0.09	1.851·10 ^{−13}	0.858	4.8	11.08	8.5·10 ^{−5}	1.9·10 ^{−5}	0.992
M1pf	4.35 ± 0.37	25.35 ± 1.21	597.38 ± 7.53	2.37 ± 0.08	3.31·10 ^{−13}	0.989	1.4	3.6	3.1·10 ^{−5}	6.8·10 ^{−6}	0.987
M2pf	4.12 ± 0.28	26.48 ± 1.35	583.21 ± 6.51	2.38 ± 0.11	5.22·10 ^{−13}	0.890	4.3	8.8	4.8·10 ^{−5}	1.8·10 ^{−5}	0.990

WS is considered an important parameter related to the biodegradability of composite structures and also to drug delivery, as most often the release mechanism is controlled by drug diffusion and matrix degradation [39]. Furthermore, water-soluble matrices improve drug delivery efficiency via transdermal formulations [40].

Water solubility was determined for the studied mono- and multilayer membranes and the obtained values are presented in Table 2. The values are higher for the systems containing larger amounts of PVA. The lowest values were obtained for multilayer films containing BCNf, as expected, and can be correlated with material hydrophilicity and the reinforcing ability of nanofibrils [41].

Barrier properties are critical parameters, as ideally a WD should allow the evaporation of exudates and, at the same time, should prevent dehydration of the wound site to facilitate the optimum healing process [42]. These parameters are influenced by materials' structure, composition, and by processing steps [43]. The water vapor transmission rate (WVTR), representing basically the amount of water vapor passing through a unit area of WD, per unit time, is the most common value characterizing WD behavior in a moist environment. There are specific values reported in the literature for WVTR. For instance, based on numerous studies, Koosehghol et al., 2017 indicate that a WD should be characterized by WVTRs in the range of 76–9360 $\text{g m}^{-2} \text{ day}^{-1}$, a rather wide range that should, however, be correlated to skin/wound conditions [42].

In our case, the obtained values, as presented in Table 2, vary from 255 $\text{g m}^{-2} \text{ day}^{-1}$, obtained for M2, to almost 600 $\text{g m}^{-2} \text{ day}^{-1}$, obtained for M2pf. Analyzing the results, there are some conclusions to be emphasized: (i) WVTR depends on films structures, with much higher values for multilayer systems; (ii) NfBC addition in the outer layer determined improved permeability due to its high hydrophilicity; (iii) an increase of TC-HCl concentration did not significantly affect the WVTR, but a slight reduction determined by concentration increase can be noticed, as reported for other membranes with microscopic porosity [43].

Overall, considering the obtained results, we can conclude that our materials, in general, and the multilayer films especially, could be used as WD for low exudates [44].

Drug release was investigated in an aqueous environment. Fractional mass release versus time, for two levels of TC-HCl concentration is presented in Figure 2 for mono- and multilayer membranes. Defined as the amount of TC-HCl released at time τ , divided by the total amount of antibiotic released at infinite time, M_τ / M_∞ , the fractional mass release increased asymptotically to 1 for monolayer membranes.

The experimental data show a much faster release from monolayer membranes. As reported by others, there could be observed an initial faster release followed by a gradual, slower release [2]. The TC-HCl diffusion process reached the equilibrium after approximately 18 h in case of monolayer films, and after 4 to 5 days for multilayer films.

Thus, in the case of monolayer films, the release dynamics is similar in both cases, disregarding TC-HCl concentration. The entire TC-HCl quantity is released because of matrix characteristics, especially microscopic porosity generated by NpBC and TC-HCl addition. Our findings are in agreement with previously reported studies of BC-PVA matrices, showing that the addition of BC promotes less compact structures with a lower density [45,46]. Song et al., 2021, using BC powder, also observed that the structure of

BC/PVA hydrogels present a porous structure, which further modifies in the presence of the antimicrobial agent [47].

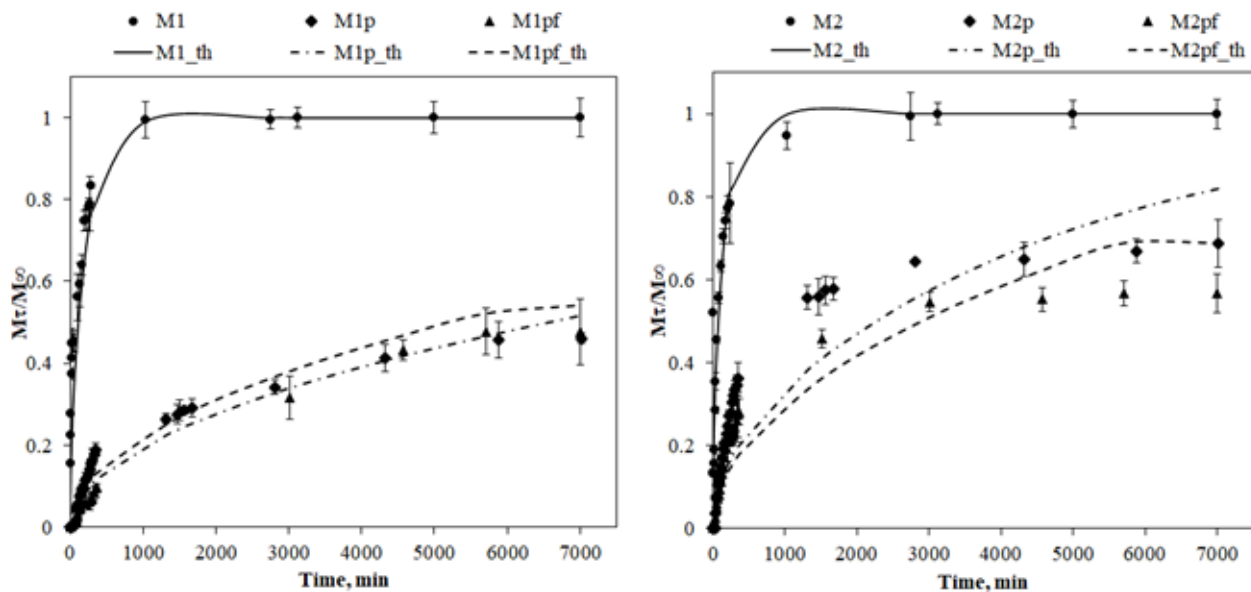


Figure 2. TC-HCl release profiles from mono- and multilayer membranes: experimental (points) and theoretical using Fick's diffusion equation (continuous lines for monolayer membranes, and dashed lines for multilayer membranes).

This proves that a complete and relatively fast release of TC-HCl from monolayer systems could be the result of the combined phenomena of polymeric matrix structural relaxation and degradation, on the one hand, and the consequence of a shorter diffusion path, on the other hand (thinner membranes).

For all obtained systems, the values of diffusion coefficients, calculated with the early-stage method (Equation (6)) based on the experimental release values, are very low, from 1.029×10^{-13} for M2 to 5.22×10^{-13} for M2pf. Similarly, low values were obtained for nisin release from multilayer antimicrobial films [33]. This could be mainly related to large TC-HCl molecules, disregarding the differences between the release systems. Furthermore, the dimension of TC-HCl molecules could explain partial drug release in the case of multilayer membranes. The decrease of the release rate could also be a consequence of polymeric structure deformation and of structural porosity modification in time, due to membrane relaxation/swelling. The experimental data were analyzed using Equation (5). The release profiles, presented in Figure 2, obtained by mathematical modeling are in good agreement only in the case of monolayer membranes, and in an acceptable agreement in the case of multilayer membranes with low tetracycline concentration.

The So and Macdonald kinetic model was applied to fit experimental data, as presented in Figure 3, in terms of TC-HCl concentration in the aq. media in time. This two-step kinetic model, traditionally used to characterize oil solvent extraction, and more recently, for the extraction or recovery of different natural components, involves two simultaneous processes, namely washing, characterized by a high rate of mass transfer, and diffusion, having lower mass transfer rates [48–50]. Each process is characterized by corresponding kinetic coefficients [35].

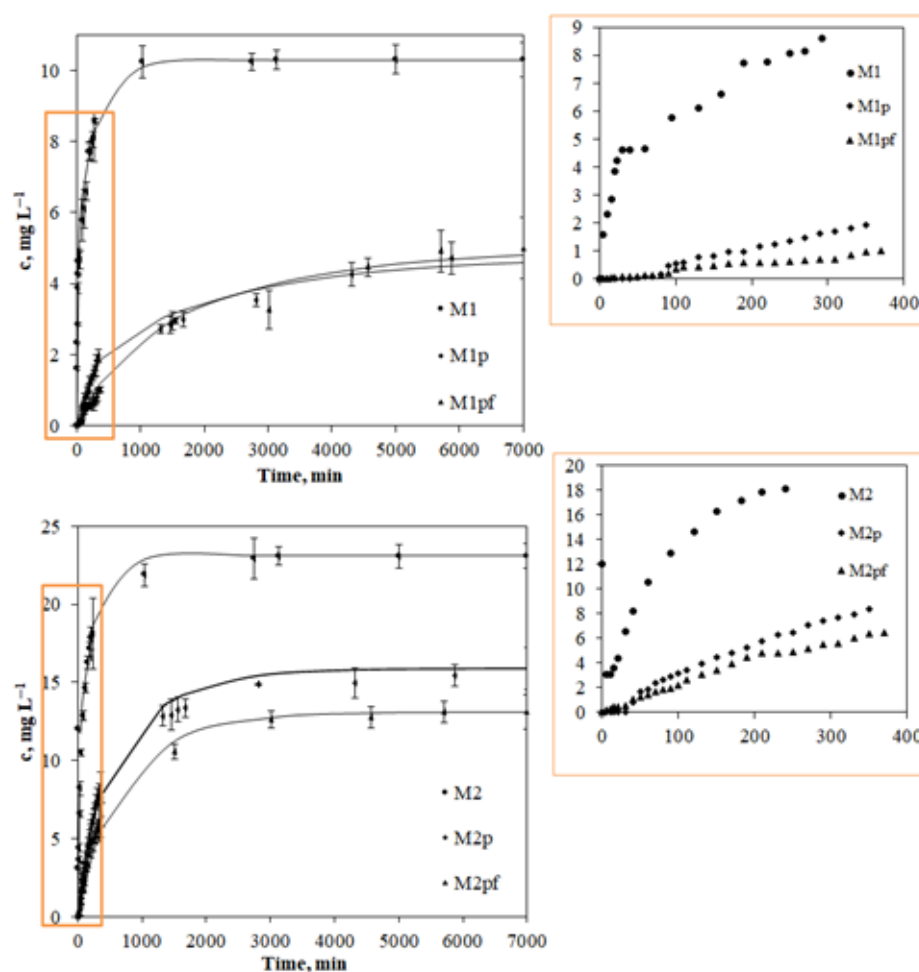


Figure 3. TC-HCl concentration in time for mono- and multilayer membranes: experimental (points) and modeled using the So and Macdonald equation (continuous lines). The inserts represent the experimental data obtained in the first 400 min.

So and Macdonald's model parameters related to TC-HCl release in an aq. environment, obtained based on experimental data, are presented in Table 2. The correlation coefficients and graphical data (continuous lines in Figure 3) show that this model is fitting much better than the experimental data. The values of k_w are higher than k_d , which confirms, in general for all of membranes, that diffusion is slower than the washing of TC-HCl at the surface. In particular for the monolayer system, values in the range of 10^{-4} min^{-1} indicate washing and matrix degradation contribution to the overall release process.

A very important aspect of drug delivery in multilayer systems is the minimization of the initial burst release. Obtaining a controlled and sustained release profile, as in the case of multilayer membranes here, represents a major advantage reducing possible cell damages in tissue culture experiments [51]. In this respect, the influence of using two different types of BC as constituents in the polymeric structure can be observed. Thus, in the case of monolayer films when NpBC was used, a porous support structure was obtained [18], which allowed complete and fast diffusion of the active principle (approximately one day). On the contrary, when NfBC was used, the relaxation of the polymeric structure was limited, as previously demonstrated [27], determining the lowest degrees of release for several days.

As a broad-spectrum antibiotic, TC-HCl can be used against Gram-negative and Gram-positive bacteria [7]. In our study, the antibacterial effectiveness was tested on two bacteria: *E. coli* DH5K (Gram-negative) and *B. subtilis* ATCC 6633 (Gram-positive). The simple and well-known disk diffusion method, with some modifications, as described before, was

applied in vitro. The investigation was performed on freshly obtained composites before TC-HCl was release and after TC-HCl was released (samples after 6000 min of continuous release) to investigate their stability in terms of antibiotic ability. The antibacterial activity was characterized as the inhibition zone (clear zone around the samples) after 24 h of incubation, and the records are presented in Table 3 and Figure 4.

Table 3. Antibacterial activity of TC-HCl-loaded mono- and multilayer membranes.

Sample ID	Inhibition Zone (mm)			
	<i>E. coli</i>		<i>B. subtilis</i>	
	Initial	After Release	Initial	After Release
M0 (control)	0	-	0	-
M1	5.6 ± 0.2	0.7 ± 0.2	8.7 ± 0.35	0.8 ± 0.2
M2	9.2 ± 0.4	0.5 ± 0.1	14.0 ± 0.9	1.0 ± 0.2
M1p	6 ± 0.3	5 ± 0.1	11 ± 0.4	8 ± 0.2
M2p	10.5 ± 0.2	12 ± 0.2	13 ± 0.6	15 ± 0.45
M1pf	5 ± 0.3	8 ± 0.2	3.7 ± 0.2	6.6 ± 0.23
M2pf	10.6 ± 0.25	9.5 ± 0.35	8.7 ± 0.18	12 ± 0.45

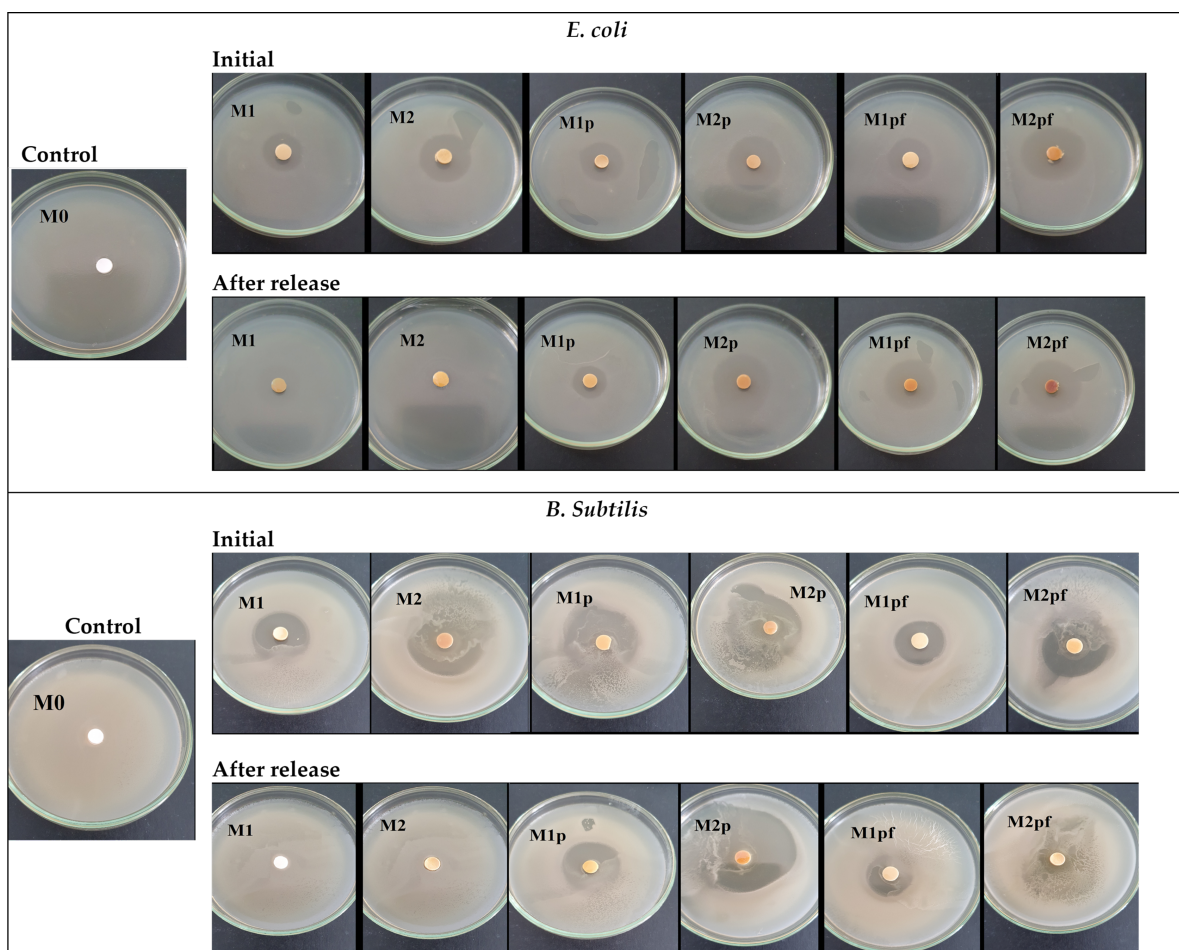


Figure 4. Antimicrobial activity against *E. coli* and *B. subtilis*.

When samples are placed on the inoculated culture media, the antibiotic diffusion occurs slower or faster depending on the composite membrane characteristics (composition, swelling ability). Then, the compound is spread on culture plates and transported across the cell membranes to be finally released into the cytoplasmic membrane, where its activity

may be expressed. Given this complex mass transfer path, and the results obtained when composites were characterized, there are few general observations that can be mentioned: (i) in all cases, the antimicrobial activity is directly correlated with TC-HCl loading, as higher antibiotic content determined a larger inhibition zone; (ii) for all samples, the inhibition zones were larger in the case of *B. subtilis*, which could be correlated with bacterial cell envelope specific to Gram-positive bacteria, which lacks an outer lipopolysaccharide membrane [52]; (iii) the monolayer composites have antimicrobial activity only in their initial form, but given their almost complete TC-HCl release, only a residual antibacterial activity was expected for the samples after release; and (iv) multilayered composites showed similar or higher antibacterial activity before and after TC-HCl release. The results are not as one can expect, but there are two factors to be considered: (a) multilayered membranes' fractional mass release after 6000 min is lower, or around 0.6, meaning almost half of the antibiotic is still loaded into membranes; and (b) during release time, the polymeric matrix is slowly degrading, allowing a more facile TC-HCl release. Nevertheless, the results show that the obtained multilayered composite matrices support drug activity and prevent its degradation for days during the release process.

The data presented in this study represent mean $\pm$ standard deviation (SD), if not stated otherwise, for experiments performed in triplicates. The statistical significance of the differences between experimental groups was calculated using one-way analysis of variance with Tukey's multiple comparison test. The values of $p < 0.05$ were considered statistically significant.

4. Conclusions

From this study, it can be concluded that mono- and multilayer composite membranes based on PVA, nano-powdered BC, and nano-fibrillated BC, and TC-HCl loaded, show good swelling abilities, and could sustain drug delivery for a few days. Moreover, the multilayer systems proved to have suitable permeability to be used as WD.

Based on the obtained experimental data and given the mathematical modeling results, a few conclusions can be highlighted: (i) all systems are diffusion-controlled systems, since the rate limiting step is drug diffusion; (ii) in the case of monolayer systems, Fick's law of diffusion could be used to characterize drug release (Fickian diffusion), and the systems works as diffusion-controlled reservoirs; (iii) multilayered systems can be regarded as membrane-controlled systems, where a thin layer of polymeric matrix covers the drug-containing matrix; (iv) the drug rate released is influenced by membrane thickness, structure, and drug molecular size; and (v) as shown from the So and Macdonald model parameters, diffusion is slower than the washing, so, the overall mass transfer rate is controlled by diffusion.

Further tests need to be carried out, in vitro and in vivo, for a deep understanding of drug release mechanisms in relation to water migration and drug diffusion through complex membrane systems capable of high swelling degrees. Overall, the obtained membranes could be promising systems for sustained drug delivery applications in healthcare, as their stability and antibacterial activity in time was proven.

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