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**POLYSULFONIC MATERIALS WITH BIOLOGICALLY
ACTIVE PROPERTIES**

SUMMARY OF DOCTORAL THESIS

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We inform you that on **November 25, 2025**, at **11:00**, in the **Conference Hall of the "Petru Poni" Institute of Macromolecular Chemistry** in Iasi, the public defense of the doctoral thesis entitled "*Polysulfonic materials with biologically active properties*" by Oana Dumbrava, will take place, to achieve the scientific title of doctor.

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INTRODUCTION

Polysulfones are a class of high-performance thermoplastic polymers with remarkable properties. They are distinguished by excellent thermal stability, maintaining their structural integrity and performance even at high temperatures, an essential aspect for processes such as autoclaving sterilization, indispensable in the medical field. In addition, polysulfones exhibit remarkable chemical stability, which gives them resistance to a variety of acids, bases and solvents, ensuring durability of the material in the presence of biological fluids or disinfectant solutions. Furthermore, polysulfones are distinguished by high mechanical strength and intrinsic biocompatibility. The latter is a fundamental property, indicating that the material does not cause adverse or toxic reactions by interacting with living tissues or blood.

Due to these exceptional characteristics, polysulfones are frequently used in various fields of biomedical engineering. One of the most important applications of this polymer is the manufacture of membranes for dialysis and blood oxygenation. In dialyzers, the controlled pore structure of polysulfones allows for the efficient filtration of toxins from the blood, while in blood oxygenators it facilitates gas exchange, both processes being vital for life support. In addition, due to their mechanical strength and ability to be repeatedly sterilized, polysulfones are optimal materials in the manufacturing processes of surgical instrument components, such as handles or housings, ensuring durability and compliance with strict hygiene standards. Polysulfones are also studied in the field of tissue engineering, to create three-dimensional support structures (scaffolds), which provide a stable and compatible matrix for cell growth and differentiation.

Although polysulfones have considerable application potential, especially as biomaterials in various medical devices and techniques, research aimed at further exploring their properties and developing new biological applications remains limited. The absence of comprehensive studies and specialized literature underscores a substantial opportunity for continued investigation. Therefore, there is both a favorable framework and a clear need for new studies aimed at advancing fundamental and applied research in this field.

For this reason, the present thesis seeks to make a significant contribution to advancing the applicability of polysulfone materials in the biomedical field by addressing three major objectives:

- Generating a fundamental framework for understanding the influence of the structure of polysulfones on their properties, by investigating their behavior in solution, in order to obtain useful information regarding the preparation of films/coatings for medical devices;
- Developing drug delivery systems based on polysulfone derivatives;
- Improving the properties of polysulfones by functionalization with a biopolymer, chitosan, and evaluating the impact of the chemistry, the obtaining process or the nature of the material on the performance.

The importance of the doctoral thesis lies in the potential of the research undertaken aiming to ***increase therapeutic efficacy through local administration*** (via controlled and targeted release, the drug reaches the target tissue in optimal concentration, minimizing systemic side effects), ***reduce the frequency of administration*** (sustained-release systems can reduce the need for repeated administration of doses), and ***develop multifunctional materials*** (by using components that present complementary activities, for example antimicrobial and antioxidant).

Thus, the novelty of the present thesis consists in exploring the ways of functionalizing the polysulfone structure, as well as in the rational and multifunctional design of the obtained systems, thus offering different strategies for controlling the properties of the material (biocompatibility, broad-spectrum antimicrobial activity, controlled drug release, antioxidant activity, hemostatic properties), adapting them to specific requirements in the biomedical field.

The doctoral thesis entitled "**Polysulfone materials with biologically active properties**" had as its main goal the exploitation and improvement of the properties of polysulfones to meet stringent needs in the biomedical field. In this regard, commercial polysulfone was purified and chemically modified to obtain polysulfone derivatives capable of generating advanced materials with applicability in the biomedical field.

The doctoral thesis is divided into two main sections. The first part (**Chapter 1**) includes a review of specialized literature, while the second part (**Chapters 2-5**) contains the results of the original contributions of this thesis.

Chapter 1 provides a review of the literature, detailing the current state of research in the field of polysulfone and materials obtained from this polymer, with a focus on properties, chemical functionalization methods and applicability in the biomedical field.

Chapter 2 is organized into two subchapters represented by two distinct and at the same time interconnected studies. Both subchapters begin with a brief introduction highlighting the reasoning behind the studies and a justification regarding the novelty brought to the field. The *first subchapter* aims to investigate the structure-properties-processing relationship of two polysulfone derivatives, quaternized polysulfone and sulfonated polysulfone, with the aim of designing functional systems, adapted for application as coating for medical devices. In the present study, the solubility parameter of the polymers was determined by a theoretical approach, based on the group contribution method, and an experimental approach, based on viscosimetric measurements of polyelectrolyte solutions in four different solvents. Subsequently, the compatibility of functionalized polysulfones with various solvents or solvent mixtures was evaluated based on the Hansen sphere theory, followed by investigating the behavior of these polysulfone derivatives in DMF – the solvent with the best compatibility. Their intrinsic viscosity, reduced specific hydrodynamic volume, macromolecular cluster density and B parameters were analyzed and compared, providing information about their conformation in solution and their interactions with the solvent.

The second subchapter uses the conclusions drawn from the first study in the preparation of coatings for medical instruments with dual activity: antibiotic and antifungal. In this regard, the solvent identified as optimal for the synthesized and studied polymers was used to prepare quaternized polysulfone solutions, with the aim of being used as an encapsulation matrix for two antimicrobial agents: norfloxacin, a broad-spectrum antibiotic, and amphotericin B, an antifungal drug. The systems were characterized from a structural, morphological, supramolecular point of view, and their properties (wettability, surface energy, antioxidant and antimicrobial activity) were also investigated. Last but not least, the release kinetics of norfloxacin from the obtained systems were monitored. The recorded results confirmed the theoretical design underlying the preparation of these coatings, highlighting the potential of quaternized polysulfone to be used as an encapsulation matrix for antimicrobial agents, thus obtaining materials that can be applied to the surface of medical devices to effectively prevent infections at the surgical site.

Chapter 3 aimed to obtain controlled drug release systems by grafting an antifungal drug, amphotericin B, onto the side chain of formylated polysulfone. The reaction between the polymer and the drug was carried out using different ratios of their functionalities, aldehyde and amine, respectively, thus leading to the obtaining of four imine derivatives of polysulfone, which were subsequently physically mixed with poly(ethylene glycol), a polymer often used in biologically active formulations, to obtain viscous solutions with superior film-forming capacity. The systems were characterized from a structural, thermal, supramolecular, morphological and topographical point of view. Since the intended purpose was to use the obtained films in the treatment of onychomycosis, in the form of nail polish, a series of properties were evaluated, such as: the sustained release capacity of amphotericin B, wettability and hydrolytic stability, antifungal activity on various strains of *Candida* and on *Saccharomyces cerevisiae* and biocompatibility on normal human dermal fibroblasts. Due to their effective antifungal activity and biocompatibility, these systems have high potential to be used as nail polishes in the treatment of onychomycosis.

Chapter 4 describes the preparation of hybrid systems based on polysulfone and chitosan by imination and amination reactions, on the surface or in the bulk, and their exhaustive characterization from a structural, morphological, topographical, supramolecular and thermal point of view. In order to establish their application potential, a wide range of properties were investigated, such as: wettability and surface free energy, swelling capacity in PBS, enzymatic biodegradation in the presence of lysozyme, water vapor transport capacity, antioxidant and antimicrobial activity, as well as the hemostatic effect. The results suggest that materials based on chitosan and polysulfone, linked by imine or amine units, represent a promising approach for the development of biomaterials with improved properties for biomedical applications.

The thesis concludes with **Chapter 5**, which includes the experimental support on which the obtained results are based: the solvents and reagents used for the synthesis of the compounds presented in the thesis, the synthesis procedures as well as the methods and equipment used for their characterization.

The thesis also includes a list of the results disseminated during the doctoral studies: scientific articles published or in the process of publication and participation in scientific events. The thesis concludes with the bibliographical references consulted in the development of the thesis.

2.1. Ionic polysulfone derivatives: synthesis, solubility parameters and behavior in solution**2.1.1. Introduction**

Polysulfones and their derivatives represent an important class of polymers that are distinguished by their superior structure and physicochemical properties (thermal and chemical stability, mechanical strength, film-forming properties) [1, 2]. The introduction of functional groups into the polymer structure is an optimal way to improve its properties and expand its applicability [3]. Among the numerous ways to chemically modify the structure of polysulfones, the reactions of chloromethylation, quaternization and sulfonation stand out, because they have a positive impact on the characteristics of the polymer, such as its reactivity, solubility, flexibility and hydrophilicity. Moreover, the reactions of quaternization and sulfonation of polysulfones represent an efficient method for obtaining ionic polymers (polyelectrolytes), a class of chemical compounds of great interest in various scientific fields due to their physical and chemical properties [4]. The present study adopts both a theoretical and experimental approach to investigate the structure-property-processing relationship of two ionic polysulfone derivatives, **PSFQ** and **PSFS**, with the aim of designing materials for biomedical applications. For the first time, the solubility parameters of these two polymers were determined by a dual methodology: a theoretical approach based on the group contribution method and an experimental approach based on viscosimetric measurements of polyelectrolyte solutions in four different solvents. Subsequently, their compatibility with various solvents or solvent mixtures was evaluated based on the Hansen sphere theory. After the selection of solvents, the study focused on investigating the hydrodynamic behavior of these ionic polysulfones. Their intrinsic viscosity, reduced specific hydrodynamic volume, macromolecular cluster density and hydrodynamic interaction parameter *B* were discussed and compared, providing information about their conformation in solution and their interactions. The novelty of this study lies in establishing a rational and transferable methodology for selecting compatible solvents for the two ionic polysulfones, supported by both theoretical predictions and experimental validation. In addition to providing insights into the solution behavior of **PSFQ** and **PSFS**, this study also provides a framework for the development of polysulfone-based materials, facilitating the optimization of material properties from the design stage, through strategic solvent choice, prior to fabrication.

2.1.2. Results and Discussion

A preliminary step in the synthesis of the polysulfone derivative with quaternary ammonium groups consists in the chloromethylation reaction of polysulfone with an equimolar mixture of paraformaldehyde and trimethylchlorosilane. The success of the chloromethylation reaction was demonstrated by FTIR, ¹H-NMR and ¹³C-NMR spectroscopy. In the ¹H-NMR spectrum (Figure 2.3.) of **PSFCM**, along with the characteristic signals of the protons from the aromatic nuclei of the substituted polysulfone (7.87-6.83 ppm), and the signal of the isopropyl

protons of the bisphenol fragment (1.70 ppm), a signal appears at $\delta = 4.54$ ppm, attributed to the two protons of the $-\text{CH}_2\text{Cl}$ group.

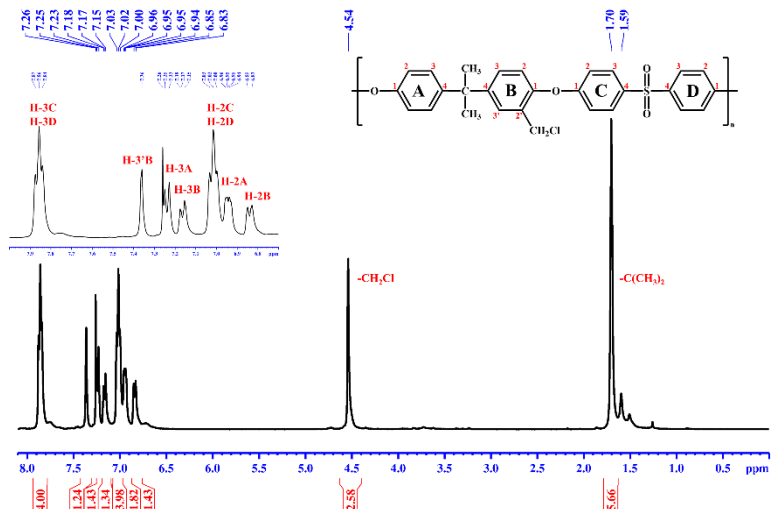


Figure 2.3. ^1H -NMR spectrum of **PSFCM-5** derivative

The introduction of quaternary ammonium groups into the polysulfone structure occurred through the reaction between the reactive chloromethylene groups ($-\text{CH}_2\text{Cl}$) on the polysulfone chain and the quaternizing agent, *N,N*-dimethylbutylamine (DMBA), in a molar ratio of 1:1.2. The functionalization of **PSF** with dimethylbutylammonium chloride was clearly demonstrated by the appearance in the ^1H -NMR spectrum of **PSFQ** of signals located at 3.28, 3.03-2.99, 1.18-1.13, 1.67 (overlapping with the signal for $-\text{C}(\text{CH}_3)_2$ from bisphenol) and 0.86-0.81 ppm, which correspond to the protons of this group. Also, the chemical bonding of dimethylbutylammonium group to polysulfone through the methylene group is also evidenced in the ^1H , ^{13}C -HMBC spectrum (Figure 2.9.) of **PSFQ** by the presence of correlation signals over two or three bonds between the C-5 carbon atom of the methylene group, located at 61.2-61 ppm, and the H-3'B proton of bisphenol A, as well as with the H-10 protons of the dimethylbutylammonium group.

Sulfonated polysulfone (**PSFS**) derivatives with different degrees of substitution were obtained by direct sulfonation of Udel polysulfone, using **chlorosulfonic acid** or a **mixture of chlorosulfonic acid and trimethylchlorosilane** as sulfonating agents, as well as by varying different reaction conditions (concentration of sulfonating agent, temperature or molar ratio between PSF and sulfonating agent). The synthetic route using an increased concentration of chlorosulfonic acid generated the best yield, leading to the obtaining of a sulfonated polysulfone derivative with a degree of substitution of 0.63, and the experiments carried out in the following studies were performed with this derivative. The sulfonated polysulfone was structurally characterized by means of FTIR, ^1H -NMR and ^{13}C -NMR spectroscopy.

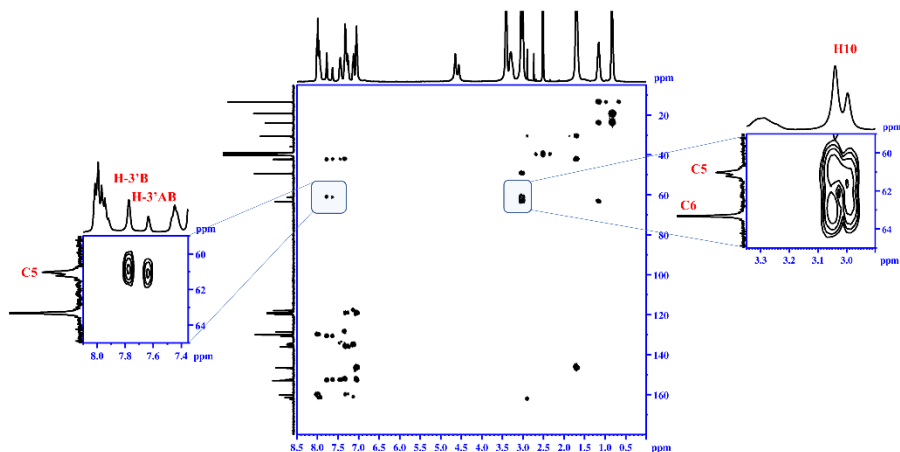


Figure 2.9. ^1H , ^{13}C -HMBC spectrum of **PSFQ** highlighting the correlation signals between the substituent and polysulfone

The relationship between the structure and thermal stability of unmodified polysulfone (**PSF**) and functionalized polysulfones (**PSFCM**, **PSFQ**, **PSFS**) was investigated using thermogravimetric analysis (TGA). According to the thermogravimetric curves shown in Figure 2.14, the thermal stability of the studied compounds decreases in the following order: **PSF**>**PSFCM**>**PSFS**>**PSFQ**, which suggests that the chemical modification of polysulfone with bulky side groups leads to derivatives more susceptible to thermal degradation, these functional groups having a spacer effect between the macromolecular chains and disrupts the intermolecular forces between them.

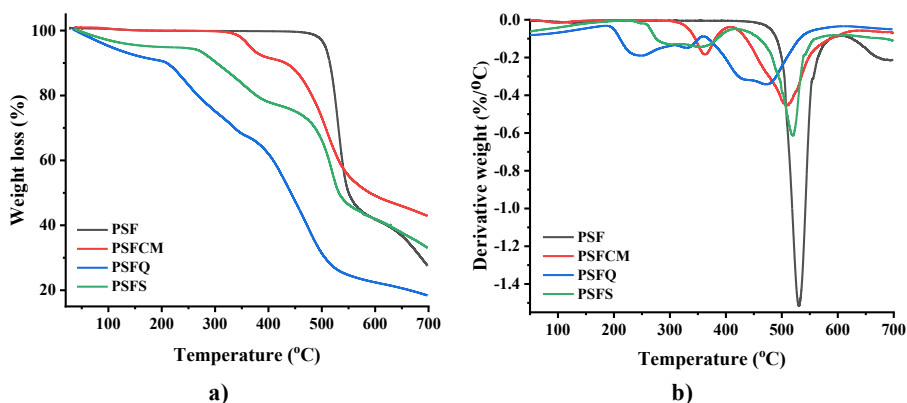


Figure 2.14. a) TG and b) DTG curves recorded for unmodified polysulfone and polysulfones functionalized with chloromethyl (**PSFCM**), dimethylbutylammonium chloride (**PSFQ**) or SO_3H (**PSFS**) groups

For a more accurate classification of the solvents and solvent mixtures investigated in this study, the components of the Hansen solubility parameters of the functionalized polysulfones,

determined using the **improved group contribution method**, were correlated with the components of the Hansen solubility parameters of the analyzed solvents, according to the **Hansen solubility sphere theory** (Figure 2.15.). The three-dimensional representation of the Hansen solubility parameters can be observed in Figure 2.15. Theoretical methods for estimating the solubility parameter (group contribution method and Hansen solubility sphere) of quaternized and sulfonated polysulfone revealed that the two polymers present values close to those of the solvents DMSO, DMAc, DMF and NMP, which is why they can be considered good solvents for functionalized polysulfones from a thermodynamic point of view. However, their intrinsic viscosity, $[\eta]$, presented the highest values when the polymers were dissolved in DMF, which denotes that this solvent induces an increase in the hydrodynamic dimensions of **PSFQ** and **PSFS**, as supported by Flory's theory [5]. Furthermore, analyzing the topography of the polymer films obtained by solubilizing the polymers in the four solvents identified as the most compatible with functionalized polysulfones, it was observed that the films prepared using DMF as solvent present a smoother surface, recording lower values of average roughness (S_a) and root mean square roughness (S_q). Thus, analyzing the results of the theoretical and experimental studies presented in this chapter, it can be concluded that *N,N*-dimethylformamide is the optimal solvent for quaternized and sulfonated polysulfones, which is why the following studies were performed using this solvent.

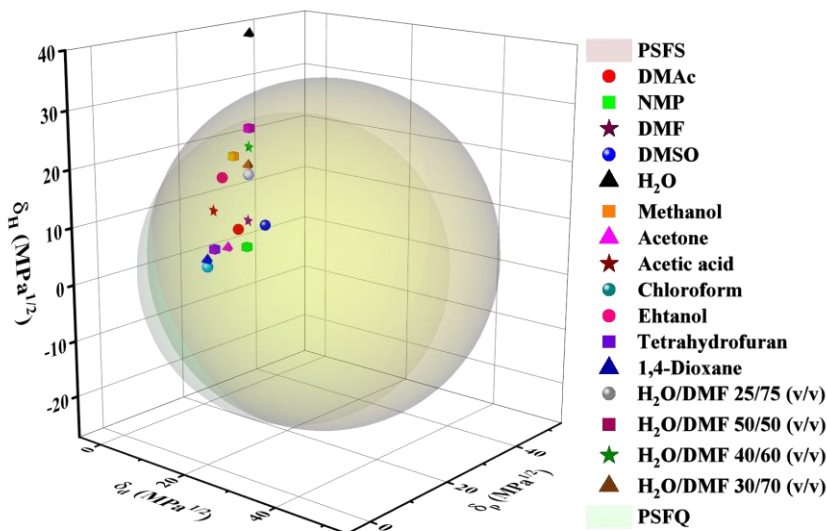


Figure 2.15. 3D representation of the partial solubility parameters corresponding to dispersion (δ_d), polar (δ_p), and hydrogen bonding (δ_H) forces, according to Hansen's solubility sphere theory, for the polysulfones studied in various solvents/solvent mixtures

The experimental method by which the behavior and properties of the synthesized polymers in solution were discussed was viscometry. While various equations are cited throughout the literature for determining the intrinsic viscosity, $[\eta]$, the Huggins equation is most frequently employed in practice [6]. The dependence of η_{sp}/c on the polymer concentration indicates that the chemically modified polysulfones, **PSFQ** and **PSFS**, exhibit a specific polyelectrolyte behavior in dilute solution, as a result of the Coulombic repulsions between the localized charge groups. Thus, the reduced viscosity increases as the polymer concentration decreases due to the increase in the macromolecular coil size (Figure 2.21).

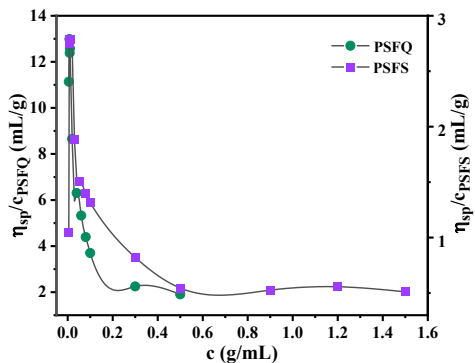


Figure 2.21. Huggins plot for **PSFQ** and **PSFS** in DMF at 25 °C

Due to the impossibility of evaluating the intrinsic viscosity by extrapolation to infinite polymer concentration, the values of this parameter of **PSFQ** and **PSFS** were determined by applying the Wolf method [7]. Figure 2.22. illustrates the progressive increase in the relative viscosity values with increasing polymer solution concentration over the entire temperature range. As the concentration increases, the differences become more pronounced, due to the fact that the increasing number of polyelectrolyte molecules increases the ionic strength of the solution and the effects of Coulombic interactions are established.

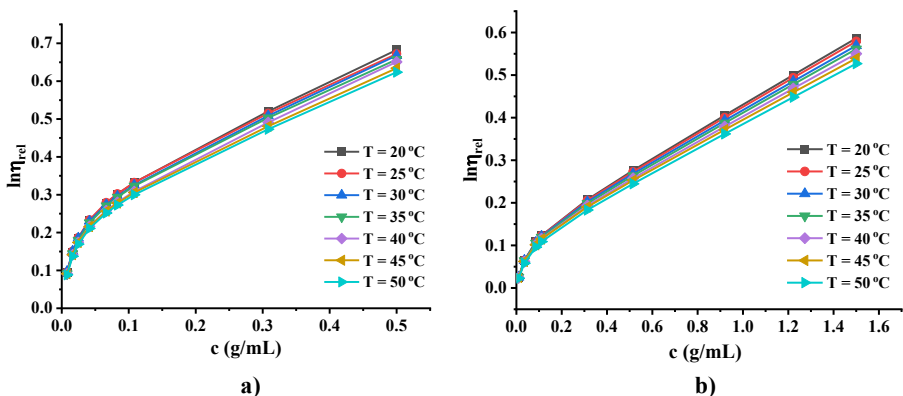


Figure 2.22. Dependence of $\ln \eta_{rel}$ on the polymer concentration, c , for a) **PSFQ** and b) **PSFS** solutions in DMF, in the temperature range 20÷50 °C

To further understand the viscosimetric behavior of functionalized polysulfones in dilute solutions, other hydrodynamic parameters were investigated, such as the reduced specific hydrodynamic volume, $\{\eta\}/[\eta]$, which provides information about the changes in the hydrodynamic volume of an individual macromolecular chain induced by the presence of other macromolecular chains nearby. The graphical representation of the dependence of $\{\eta\}/[\eta]$ on

$c[\eta]$ of **PSFQ** and **PSFS** indicates a decrease in the specific hydrodynamic volume of polymers in DMF with increasing reduced polymer concentration, over the entire range studied (Figure 2.23.). This behavior was correlated with the influence of charge density and counterion dissociation on the conformation of polyelectrolyte chains. Thus, at high dilution, the counterions are far from the polyion, and the chains become more extended due to the ionic repulsion forces between the charged groups of the macroion. According to this approach, as the polymer concentration increases, the polyelectrolyte coil shrinks due to progressive charge screening.

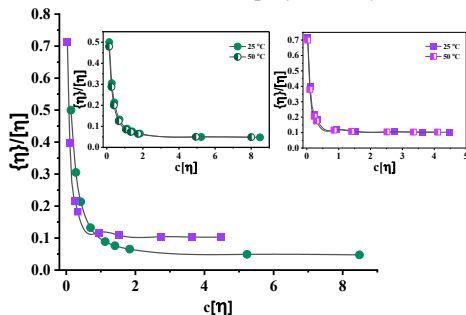


Figure 2.23. Variation of the reduced specific hydrodynamic volume ($\{\eta\}/[\eta]$) as a function of the reduced concentration ($c[\eta]$) for **PSFQ** (●) and **PSFS** (■) polymers, at 25 °C. The inset images show the same variations at different temperatures

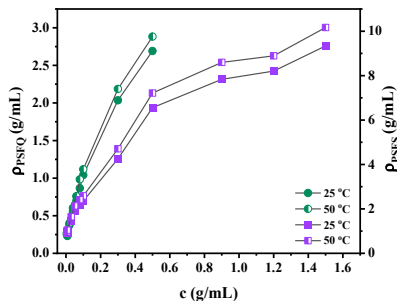


Figure 2.24. Variation of macromolecular coil density as a function of polymer concentration for **PSFQ** (●) and **PSFS** (■) in DMF at 25 °C and 50 °C

The variation of the reduced specific hydrodynamic volume as a function of the reduced polymer concentration can be correlated with the dependence between the density (ρ) and the macromolecular coil as a function of concentration (Figure 2.24.). As the polymer concentration increases, the polyelectrolyte coil shrinks due to the progressive screening of charges and the increase in intramolecular interactions. For this reason, the density of the macromolecular coil in both the case of **PSFQ** and **PSFS** increases slowly with increasing the polymer concentration. However, we can observe that the values of this hydrodynamic dimension in the case of **PSFS** are higher than those of **PSFQ**, which suggests that DMF is less good thermodynamic solvent for **PSFS** than for **PSFQ**, the expansion of the macromolecular chain induced by the solvent being less pronounced [8].

2.2. Quaternized polysulfones as matrices for the development of broad-spectrum antimicrobial coatings for medical devices

2.2.1. Introduction

In recent decades, medical devices, especially those for the diagnosis, monitoring and treatment of injuries or diseases, have made a remarkable contribution to improving and increasing the life expectancy of people around the world [9, 10]. However, their use also represents a means of transmitting nosocomial infections, which endanger the health and, in some

cases, the lives of patients [11, 12]. The development of coatings that can be easily applied to the surface of medical devices and that prevent the adhesion and growth of bacteria, without affecting the tissue with which they come into contact, represents a promising solution that can prevent the occurrence of infections and improve the life quality of patients. In this context, to address the persistent challenges generated by infections associated with medical devices, especially in clinical and surgical environments, the working hypothesis of this study was formulated towards the development of coatings that not only inhibit the growth of bacteria and fungi, but also actively eliminate these microorganisms. With this objective in mind, the present study explores the use of quaternized polysulfone as a matrix for the encapsulation of two antimicrobial agents: norfloxacin (NFX), a broad-spectrum quinolone antibiotic, and amphotericin B (AmB), a polyene antifungal drug. This approach aims to create coatings with enhanced antibacterial and antifungal properties, specifically designed to prevent biofilm formation on surfaces of medical devices. The coatings were designed based on a rational, multi-level strategy, taking into account: (i) the ability of quinolones to enhance the efficacy of AmB through synergistic effects, as previously demonstrated in some studies [13], (ii) the chemical structures of the polymer and the two drugs that favor strong electrostatic interactions, capable of supporting the controlled release of drugs, (iii) the complementary bioactivities of the antimicrobial agents, NFX having antibacterial effects and AmB antifungal action, their combination in the same material leading to coatings with broad-spectrum antimicrobial activity, and (iv) the inherent antimicrobial activity of the quaternized polysulfone matrix, with the potential to effectively inhibit a wide range of pathogenic strains, thus enhancing the overall antimicrobial efficacy of the coatings.

2.2.2. Results and Discussion

Comparative analysis of the FTIR spectra obtained for the drug-containing formulations, along with their reference components (polymer matrix and individual drugs) revealed slight variations in both the positions of the absorption bands and their intensities. Thus, the broad band in the FTIR spectrum of the **PSFQ** sample, located at 3395 cm^{-1} , progressively shifts to lower wavenumbers with increasing amounts of encapsulated drugs. This trend suggests the formation of strong intermolecular interactions between the polymer matrix and the encapsulated drugs. Considering the chemical structure of the incorporated drugs, which contains carboxylic groups, hydroxyl and primary amino groups in the case of AmB, but also ketone, secondary and tertiary amino groups and fluorine atoms in the case of NFX, it is plausible that these interactions involve both hydrogen bonds and electrostatic forces, which contribute to the observed spectral shifts.

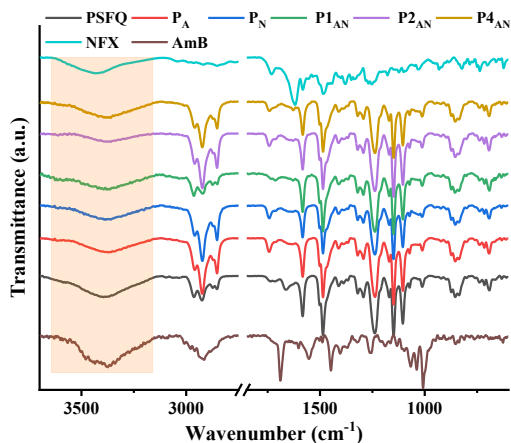


Figure 2.28. FTIR spectra of the investigated coatings, as well as of the two drugs

Supramolecular characterization of the formulations was performed using wide-angle X-ray diffraction. The diffraction patterns of the formulations are very similar to that of **PSFQ**, without distinct reflections specific to AmB or NFX. This suggests that the drugs were finely dispersed in the polymer matrix, probably due to intermolecular interactions with **PSFQ** that inhibit crystallization, at least below the detection level of this technique [14]. However, a slight shift of the diffraction peaks to higher degrees and consequently smaller intermolecular distances was observed, suggesting that the presence of the drugs promoted the coalescence of the polymer chains into more compact ordered domains. This effect is supported by the appearance of more pronounced birefringent domains under polarized light [15]. The lack of clear diffraction bands characteristic to crystalline drugs reinforces the idea of a very fine dispersion of the drugs in the polymer matrix.

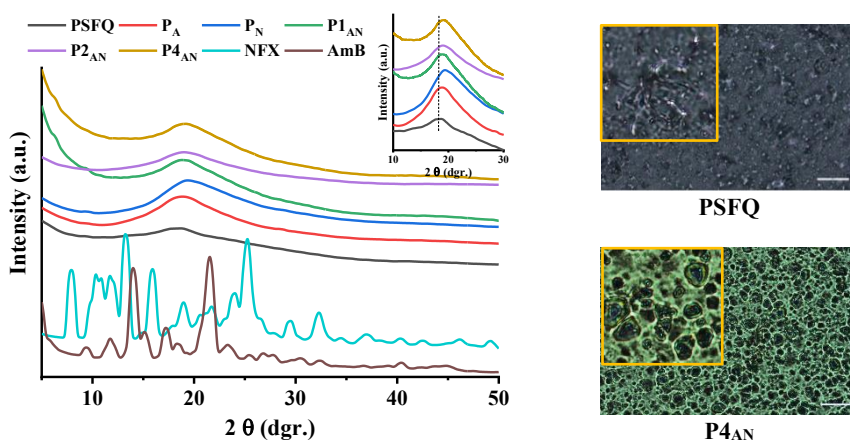


Figure 2.31. XRD diffractograms of **P_A**, **P_N**, **P_{1AN}**, **P_{2AN}**, **P_{4AN}** coatings and of the control components **PSFQ**, AmB and NFX and POM images of the **PSFQ** film, as well as of the sample containing both drugs (**P_{4AN}**)

The surfaces of the coatings containing one or both drugs have water contact angle values in the range of 60-90°, associated with good biocompatibility. In addition, moderate wettability is crucial for optimal interaction with water, facilitating efficient drug release and increasing its bioavailability [16].

The drug release kinetics were monitored *in vitro* in PBS (pH = 7.4). The samples demonstrated a similar capacity to release NFX (between 75 % and 77 %) over 48 hours, with a faster release in the first 4 hours (Figure 2.33. a, b). The drug release rate was dependent on the amount of antimicrobial agent in the matrix. Thus, sample **P_{4AN}** released 69 % of the encapsulated drug in the first 4 hours, while samples **P_N** and **P_{1AN}** released only ~51 %. Monitoring the kinetics of AmB release from the polymer matrix was not possible, the supernatant concentration falling below the detection limit of the UV-Vis spectrophotometer. Following the fitting of the experimental release data to different mathematical models, it was found that the release of NFX from the polymer matrix is a diffusion-controlled process and influenced by the amount of drug, the exposure time and the hydrophilicity of the matrix.

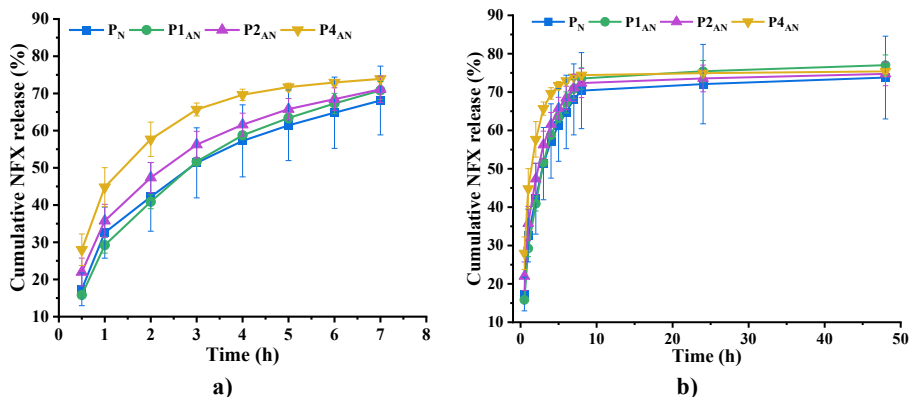


Figure 2.33. Release kinetics of NFX from P_N , $P1_{AN}$, $P2_{AN}$ and $P4_{AN}$ samples for a) 8 hours and b) 48 hours

The ability of the investigated coatings to capture reactive oxygen species (ROS) was analyzed by DPPH method (Figure 2.34). The antioxidant activity was superior in the case of samples containing AmB and increased with increasing its concentration, reaching a threshold at 67.49 %. The samples containing both drugs showed a higher antioxidant activity compared to those containing a single drug at the same concentration, indicating the cumulation of their effects. The highest antioxidant activity was observed in the case of the $P4_{AN}$ sample, 67.49 %, this result being correlated with the additive antioxidant effect of the two drugs dispersed in the polymer matrix.

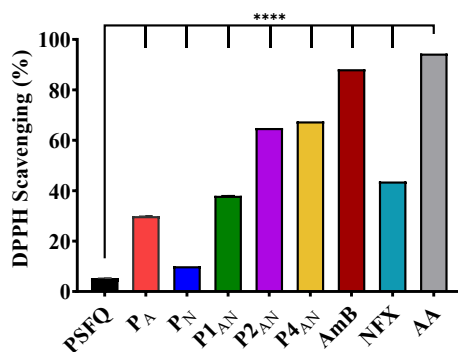


Figure 2.34. a) Antioxidant activity of the samples investigated in this study, along with 3 control samples (amphotericin B, norfloxacin and ascorbic acid-AA)

Given that the aim of this study was to develop new antimicrobial coatings, through an intimate dispersion of two drugs in a quaternized polysulfone matrix, the efficiency of the formulations was investigated against microorganisms frequently associated with nosocomial infections, namely *S. aureus* (Gram-positive bacterium), *E. coli* (Gram-negative bacterium) and *C. albicans* (opportunistic microorganism). **PSFQ** exhibited moderate efficacy against *S. aureus* (inhibition diameter up to 14 mm) and stronger antibacterial activity against *E. coli* (up to 23 mm), but did not exhibit antifungal activity. Encapsulation of AmB in the P_A sample induced antifungal activity (inhibition diameter up to 18 mm) but no antibacterial activity was detected. In contrast, encapsulation of NFX in the P_N sample significantly improved antibacterial activity compared to the **PSFQ** sample, consistent with the observed release of NFX. When both antimicrobial agents were co-encapsulated, the resulting coatings exhibited dual antimicrobial

activity, with inhibition diameters comparable to those of the P_A and P_N samples. This confirms that both drugs were released in sufficient concentrations to exert an inhibitory effect on the growth of microorganisms.

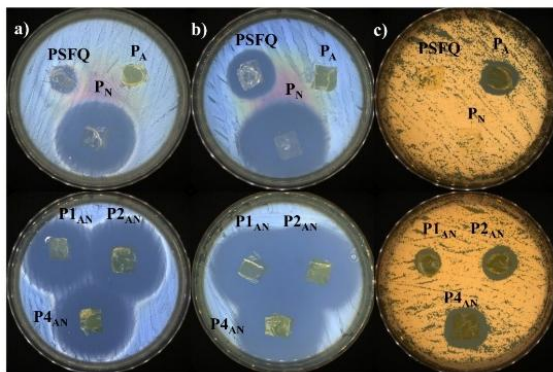


Figure 2.36. Antimicrobial activity of the investigated samples against: **a)** *S. aureus*, **b)** *E. coli* and **c)** *C. albicans*

Chapter 3

Dynamic controlled release systems based on polysulfone formylated and amphotericin B for the treatment of onychomycoses

3.1. Introduction

Onychomycosis, the medical term for nail infections, is a prevalent condition, usually caused by dermatophyte fungi, yeasts (such as *Candida* species), and non-dermatophyte fungi [17]. These microorganisms enter through minor trauma to the nail or cuticle and subsequently develop due to favorable temperature and humidity conditions. Antifungal nail polishes (also known as medicated nail polishes) are a key topical treatment option for onychomycosis, especially for superficial infections or as an adjunct to oral therapies in more severe cases [18]. Their main advantage lies in the direct administration of the drug to the infected nail, minimizing the systemic side effects associated with oral antifungals. However, treatment with antifungal nail polishes requires patience and consistency due to the slow growth rate of nails. For this reason, the development of nail polishes that act as a sustained drug delivery system is of interest, having both a medical and aesthetic function. A sustained release profile should bring important advantages, from efficiency to reduced treatment costs [19]. The objective of this study was to create reversible imino bonds between amphotericin B (AmB), an antifungal drug with primary amino groups, and a formylated polysulfone derivative, with the aim of obtaining dynamic materials that, under humid conditions, would allow controlled and sustained release of the antifungal drug [20]. A material obtained by this approach should have superior performance compared to formulations in which the drug is simply encapsulated and trapped in a polymer matrix by physical interactions alone.

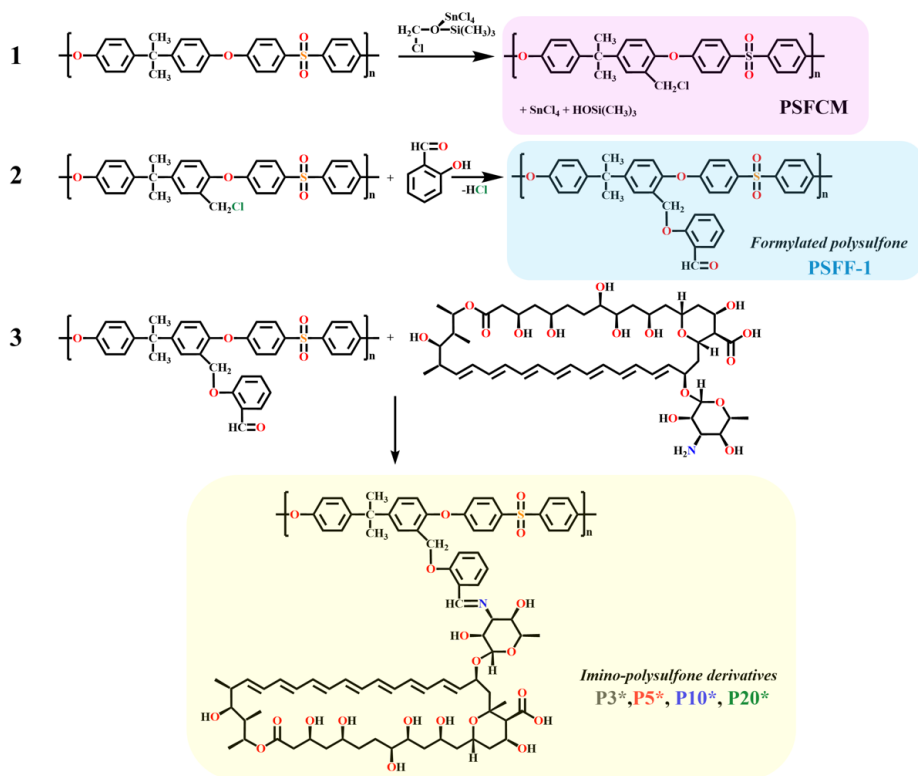
3.2. Results and Discussion

To obtain dynamic materials capable of sustained release of the antifungal drug AmB, polysulfone was modified to introduce aldehyde groups on the macromolecular chain (**PSFF-1**). For this purpose, the commercial polysulfone, previously purified, was subjected to chloromethylation and subsequently Williamson etherification (Scheme 3.1.). The obtained polysulfone with formyl groups, **PSFF-1**, was used for grafting the antifungal agent AmB by the condensation reaction of the aldehyde groups with the amino groups of the drug, in different molar ratios of their functionalities, from 3/1 to 20/1 (Table 3.1.). This synthetic route led to the obtaining of derivatives with reversible imine bonds (**P3***, **P5***, **P10***, **P20***), which in the presence of moisture can be cleaved, releasing the drug. The number in the sample code signifies the molar ratio of the CHO and NH₂ functionalities (Table 3.1.). To improve the film-forming ability of the polysulfone imine derivatives, PEG was also added to the system, and the resulting systems were coded with **P3**, **P5**, **P10**, **P20**.

Table 3.1. Composition of polysulfone imine derivatives and the obtained delivery systems

Sample	m _{PSFF-1} (g)	m _{AmB} (g)	CHO/NH ₂ molar ratio	m _{PEG} (g)	V _{DMSO} (mL)
P3	0.25	0.141	3/1	0.25	24.20
P5	0.25	0.083	5/1	0.25	22.00
P10	0.25	0.041	10/1	0.25	20.37
P20	0.25	0.020	20/1	0.25	19.29
PA	-	0.020	-	0.25	12.27
<i>Imination:</i>					
<i>P3*, P5*, P10*, P20*</i>					
				<i>Physical mixture with PEG:</i>	
				<i>P3, P5, P10, P20</i>	

P3*, **P5***, **P10***, **P20*** have the same composition as **P3**, **P5**, **P10**, **P20**, but without PEG



Scheme 3.1. Synthetic route applied in this study: **1)** chloromethylation of polysulfone, **2)** Williamson etherification reaction of chloromethylated polysulfone with salicylaldehyde and **3)** covalent bonding of formylated polysulfone with amphotericin B through imine units

The structure of the chloromethylated polysulfone (**PSFCM**), used as an intermediate, as well as that of the formylate derivative, was demonstrated by ^1H -NMR spectroscopy. The ^1H -NMR spectrum (Figure 3.1.) of the chloromethylated polysulfone displayed a signal at 4.54 ppm, assigned to the two protons of the $-\text{CH}_2\text{Cl}$ group, along with other resonance signals specific to aromatic protons, located in the range of 7.87-6.83 ppm. A singlet located at 1.70 ppm was also observed, which corresponds to the six protons of the isopropyl group in the polysulfone monomeric units. After the substitution of the chlorine atom with salicylaldehyde (SA), in the ^1H -NMR spectrum of the formylated derivative a signal appeared at 10.31-10.26 ppm, which corresponds to the aldehyde proton. In addition, the signal of the two protons of the methylene bridge was shifted to 5.13 ppm, as a result of the higher electronegativity of the oxygen atom, having a deshielding effect on the protons.

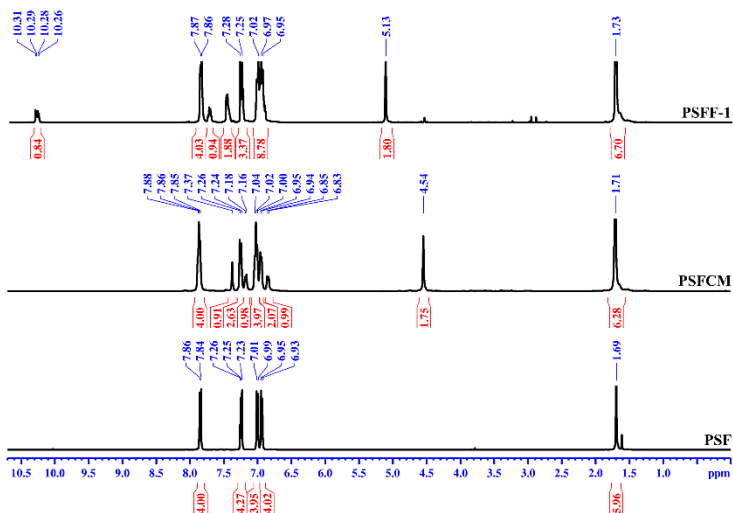


Figure 3.1. ^1H -NMR spectra of polysulfone (PSF), chloromethylated polysulfone (PSFCM) and formylated polysulfone (PSFF-1)

The NMR spectra of samples **P3***, **P5*** and **P10*** revealed the appearance of a new signal at 8.6 ppm, attributed to the imine proton (Figure 3.4.). Comparative analysis of the ratios between the integral values of the signals corresponding to the aldehyde and imine protons for samples **P3***, **P5*** and **P10*** revealed a progressive increase from sample **P10*** to sample **P3***, consistent with the increase in the amount of AmB grafted onto the **PSFF-1** chain from **P10*** to **P3***, according to Table 3.1 [20]. In the case of sample **P20***, no new signal was detected in the ^1H -NMR spectrum, most likely because, in this case, the amount of imine formed was very low compared to the high mass of the other components.

SEM micrographs of the obtained films revealed a relatively smooth surface, without cracks or visible defects.

Also, the absence of micrometric agglomerations suggests a uniform structure over the entire surface of the samples. The images obtained in cross section revealed a compact structure, with

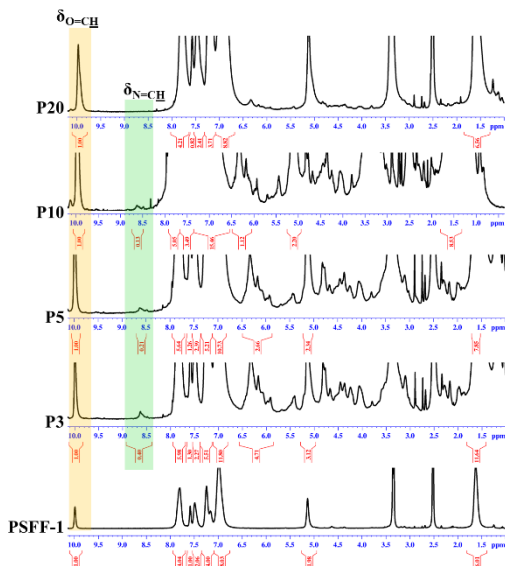


Figure 3.4. ^1H -NMR spectra of drug delivery systems

moderate roughness. By analyzing the data obtained by SEM, no significant morphological differences were observed between the analyzed samples, nor aggregations, regardless of the amount of AmB linked to the **PSFF-1** chain. This result indicates that the imination reaction, although occurring in a statistical manner, leads to the formation of polymers with a uniform morphology.

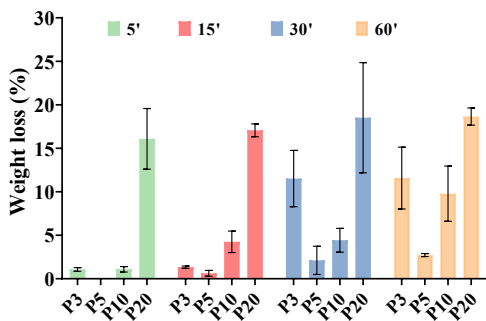


Figure 3.9. b) Hydrolytic stability of the investigated samples

The strength of the polymeric films was evaluated by estimating the percentage mass loss of the films after immersion in water for 5, 15, 30 and 60 minutes. The results revealed a decrease in hydrolytic stability after 30 and 60 minutes of immersion, with mass loss ranging from 2 to 18 % after one hour (Figure 3.9.b). The best hydrolytic stability was observed in the case of sample **P5**, while sample **P20** was the most susceptible to degradation in the presence of water. However, samples **P3-P10** showed mass losses below 10 %, in

accordance with the permitted limits (<10 % loss of the total film mass) of targeted application [21].

Given that the antifungal drug is grafted onto the **PSFF-1** chains through imine bonds, which are reversible in the presence of moisture, its release was monitored *in vitro* in PBS (pH = 7.4), for 48 hours. The samples studied released the drug at different rates, as follows: the highest release rate was recorded for sample **P20**, which contains the lowest amount of AmB, while the lowest release rate was recorded for sample **P3**, which contains the highest amount of drug. The kinetic data obtained after the *in vitro* release experiment highlighted that the grafting density of imine units onto the **PSFF-1** chains represents an important tool that can be used to adjust the release rate.

As previously mentioned, the materials were designed with the aim of obtaining bioactive coatings with antifungal properties, which could be used in the treatment of fungal nail infections. Therefore, the antifungal activity of the formulations was evaluated on several strains of fungi, usually associated with nail infection: *C. albicans*, *C. glabrata*, *C. parapsilosis* and *S. cerevisiae*. The most effective antifungal activity was observed in the case of sample **P3** against all strains tested, especially against *C. glabrata* ATCC20019 (with a diameter of the zone of inhibition of 31 mm) (Figure 3.11.a).

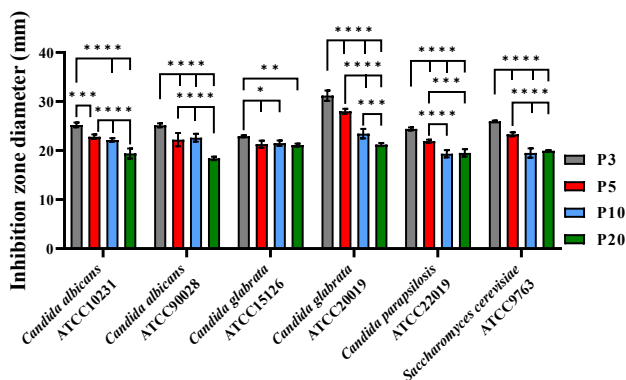


Figure 3.11. a) Antifungal activity of samples against tested strains

The cytocompatibility of the materials was evaluated according to the ISO 10993-5: Biological evaluation of medical devices – Part 5: In vitro cytotoxicity tests (2009) [22], which stipulates that a material is considered non-cytotoxic if the relative viability of cells in the presence of its extract exceeds 70 % compared to an untreated control.

Following incubation of fibroblasts with extracts obtained from the synthesized samples, the relative viability of the cells reached values higher than 82 % (Figure 3.12.). Therefore, the results of the MTS test unequivocally confirm the non-cytotoxic nature of the evaluated materials, highlighting the fact that both the materials and the components released from them do not have a negative effect on cell metabolism or proliferation *in vitro*. Furthermore, bright field cell analysis shows that the morphology of fibroblasts that were in contact with the extract from samples **P3-P20** remains unchanged compared to the control sample, presenting a fusiform shape.

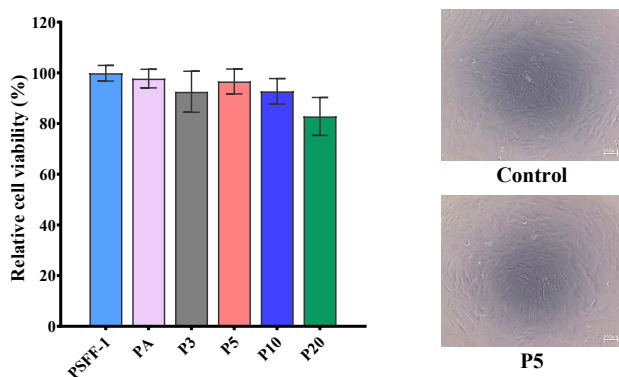


Figure 3.12. Relative cell viability after 24 hours for the extract solutions of the studied systems. Data are expressed as a percentage of the viability of the control group ($n = 9 \pm SD$). Bright field microscopy images

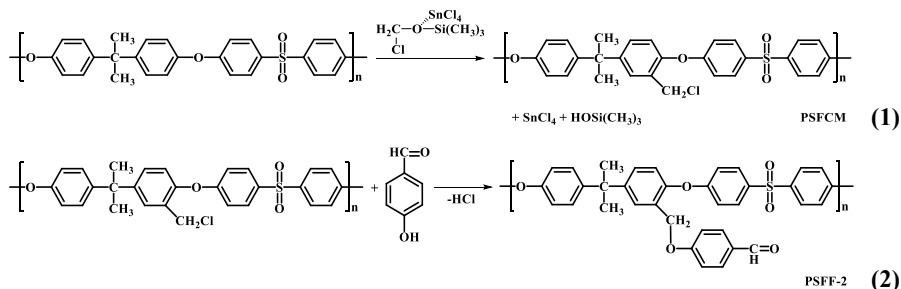
Chitosan and polysulfone-based bioactive materials obtained through imine and amine covalent bonds

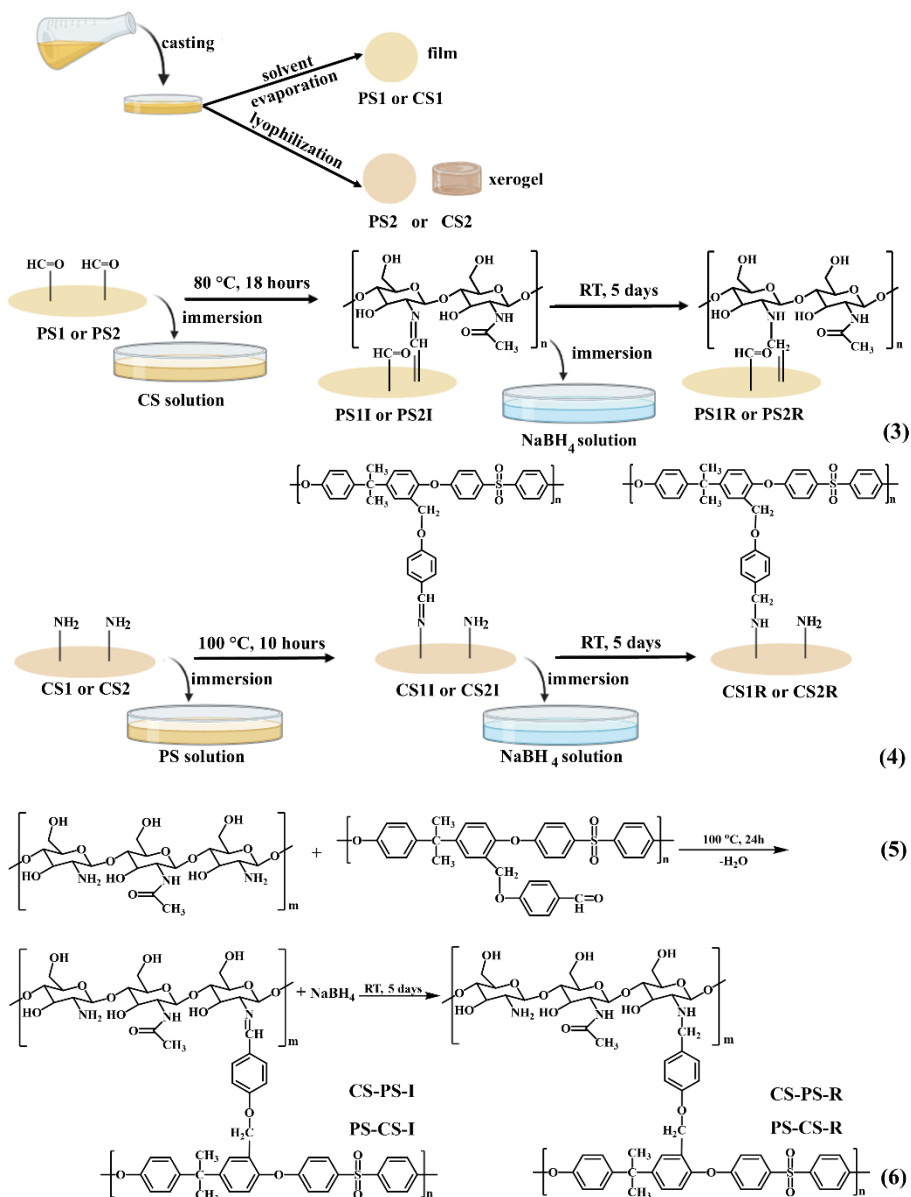
4.1. Introduction

The main properties that recommend the use of *polysulfone* in various biomedical applications, including implantable devices, biosensors and blood-contacting devices, are the chemical, thermal and hydrolytic stability, remarkable mechanical strength and high glass transition temperature [23]. Despite its excellent physical and chemical properties, the inherent hydrophobicity of polysulfone represents a major limitation, as it favors the rapid adsorption of proteins onto the material's surface, thus triggering platelet adhesion, aggregation and coagulation during prolonged exposure of these materials in contact with human blood [24]. An effective method to improve the hydrophilicity of polysulfone materials is to introduce hydrophilic organic or inorganic agents into the casting solutions [25, 26]. Chitosan, a biopolymer obtained by alkaline treatment of chitin, has received significant attention in the scientific community for its remarkable properties, such as hydrophilicity, biocompatibility and biodegradability, but also for its film-forming ability [27]. Although numerous studies have explored the modification of polysulfone with chitosan, mainly using blending or surface coating techniques [27-29], the literature to date has not documented the direct covalent bonding of the two polymers. In this context, the aim of this study was to lay the foundations for a new method of covalently bonding chitosan and polysulfone into a new material with improved properties, such as thermal stability, porous morphology, water vapor transport capacity or antioxidant activity.

4.2. Results and Discussion

A series of polysulfone and chitosan-based materials, covalently linked through imine or amine units, was prepared using different synthesis routes: **(1)** chloromethylation of polysulfone, **(2)** etherification of chloromethylated polysulfone, **(3)** grafting of chitosan onto the surface of formylated polysulfone-based materials (**PSFF-2**), **(4)** grafting of formylated polysulfone onto the surface of chitosan-based materials *via* imine linkages, **(5)** bulk synthesis of formylated polysulfone grafted with chitosan chains, **(6)** reductive amination reaction of **(3)**, **(4)** and **(5)** compounds (Scheme 4.1.).





Scheme 4.1. The main steps in the process of obtaining the studied hybrid materials and the corresponding codes: (1) chloromethylation of polysulfone; (2) etherification of chloromethylated polysulfone; (3) grafting of chitosan onto the surface of materials based on formylated polysulfone or (4) grafting of formylated polysulfone onto the surface of materials based on chitosan *via* imine or amine units; mass synthesis of formylated polysulfone with chitosan chains *via* (5) imine or (6) amine units

The results of NMR and FTIR spectroscopic analyses revealed the successful synthesis of the formyl-containing polysulfone, which was further used to react with chitosan *via* an acid condensation reaction to obtain imine units, which were subsequently subjected to a reductive amination reaction to obtain secondary amine units.

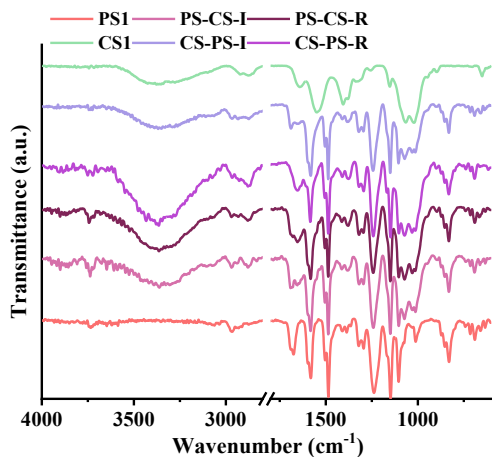


Figure 4.5. FTIR spectra of c) films prepared by the bulk imination reaction of formylated polysulfone and chitosan (**PS-CS-I**, **CS-PS-I**) and the corresponding amines (**PS-CS-R**, **CS-PS-R**)

In the FTIR spectra (Figure 4.5.) of all materials resulting from the imination of **PSFF-2** with chitosan, regardless of the method used to obtain them, either by surface imination (**CSII**, **PSII**, **CS2I**, **PS2I**) or by bulk synthesis (**CS-PS-I** and **PS-CS-I**), it was observed that the broad band specific to the vibration of the C-N amide bond in the acetylated glucosamine units of chitosan (1642 cm^{-1}) was replaced by a sharp band with an absorption maximum at 1650 cm^{-1} , most likely due to the overlap of the bands characteristic of the imine and amide groups [30]. In the FTIR spectra of the aminated materials, regardless of the preparation method, it can be observed that the absorption band characteristic

to the vibration of the imine group decreased in intensity or disappeared, suggesting partial or total conversion into secondary amine, following treatment of the investigated materials with a NaBH_4 solution.

The thermal behavior of the investigated materials was studied by thermogravimetric analysis (TGA) to evaluate the influence of chemical modification on the thermal stability (Figure 4.8.). While the control samples of chitosan and unmodified polysulfone showed characteristic degradation stages around $290\text{ }^{\circ}\text{C}$ and around $500\text{ }^{\circ}\text{C}$, respectively [31, 32], the general rule was to shift the degradation maximum towards lower temperatures, in agreement with the formation of chitosan-rich and polysulfone-rich domains. The percentage of ash residue varied between 24 % and 53 %, depending on the dominant polymer and the obtaining process.

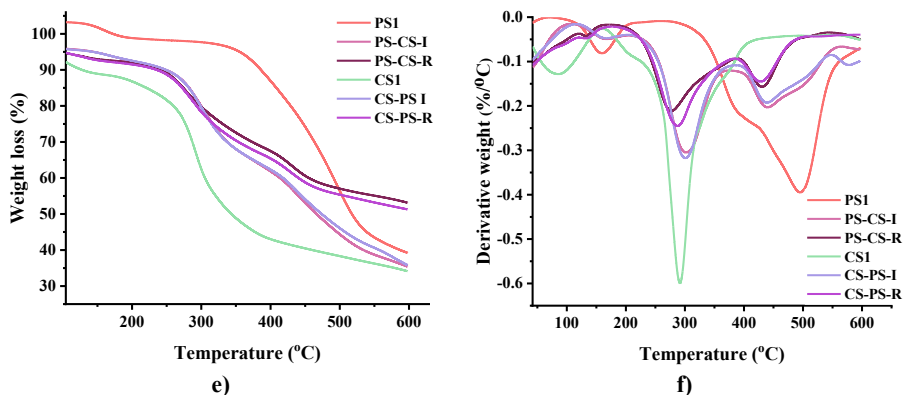


Figure 4.8. TGA and DTG curves of **e, f**) films prepared by bulk reaction of formylated polysulfone and chitosan, through imine (**PS-CS-I**, **CS-PS-I**) or amine (**PS-CS-R**, **CS-PS-R**) bonds

To further evaluate the liquid-absorbing capacity of the studied materials, their water sorption/desorption capacity was investigated, which provides information on both the water migration through their structure and the factors influencing water absorption or release [33]. The sorption/desorption curves reveal that modification of polysulfone films or xerogels with chitosan chains led to an increase in water retention from ~8 % to ~20 %, while functionalization of chitosan with **PSFF-2** led to a decrease from ~55 % to ~38 %, highlighting the role of chitosan in improving liquid retention (Figure 4.13.). In addition, bulk synthesis of **PS-CS** materials led to water absorption values between 35–45 %, suggesting that this method is suitable for controlling the material's properties. This aspect can be correlated with the disruption of the hydrophilic-hydrophobic balance of the tested materials, given the abundance of hydrophilic groups of chitosan, capable of interacting with water molecules ($-\text{OH}$, $-\text{N}=\text{CH}$, NH_2 , $-\text{NH}-$).

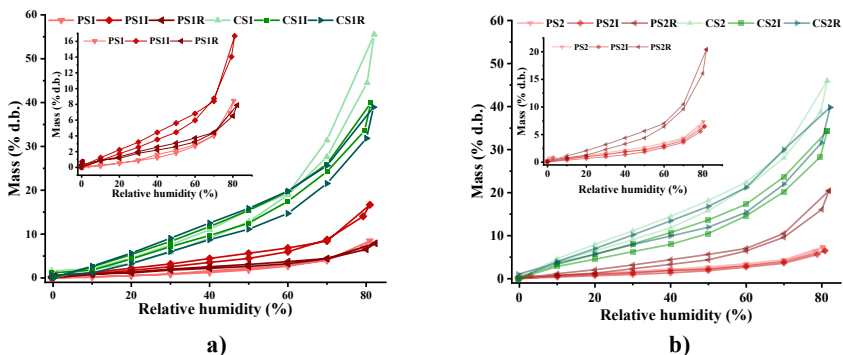
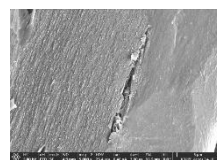
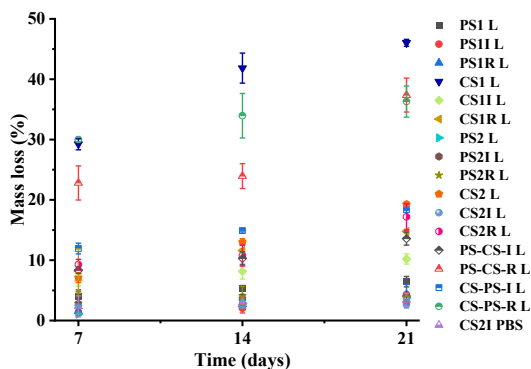
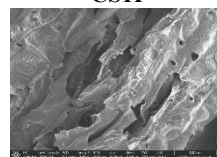


Figure 4.13. Water vapor sorption/desorption isotherms of the studied samples compared to the reference materials: **a)** polysulfone (**PS11**, **PS1R**) or chitosan (**CS11**, **CS1R**) films modified on the surface by imination or amination and unmodified polymer films (**PS1**, **CS1**); **b)** polysulfone-based xerogels (**PS21**, **PS2R**) or chitosan (**CS21**, **CS2R**) modified on the surface by imination or amination and unmodified polymer xerogels (**PS2**, **CS2**)

The biodegradation was investigated in the presence of lysozyme, an enzyme found in biological fluids, capable of cleaving the β -(1 \rightarrow 4) glycosidic bonds between D-glucosamine and *N*-acetyl-D-glucosamine units in the chitosan structure [34]. The highest mass loss was recorded for unmodified chitosan films and xerogels, 46 % and 19 %, respectively (Figure 4.15.a). By grafting polysulfone onto the surface of chitosan based materials, the degradation process was significantly slowed down, reaching mass losses of only 10 % and 2 % after 21 days of contact with lysozyme. This behavior can be explained by the formation of a hydrophobic layer after grafting formylated polysulfone onto the surface of chitosan materials, which prevents lysozyme from penetrating the material's structure. In the case of materials obtained by bulk reaction, the percentage of mass loss was higher, with values close to those of chitosan materials. This phenomenon can be explained by considering the porous nature of the materials, which favored the access of the enzyme to the chitosan domains.



CS11



CS2R

a)

b)

Figure 4.15. a) Graphical representation of the biodegradation kinetics of the investigated materials in the presence of lysozyme and **b)** representative post-degradation SEM micrographs

The samples that were immersed in the lysozyme solution were washed and lyophilized and subsequently analyzed by scanning electron microscopy, in order to observe the influence of biodegradation on their morphology. As can be seen in Figure 4.15.b, cracks appeared both on the surface of the materials and in their mass, as a result of the erosion process under the action of the enzyme, in agreement with the mass loss determined by gravimetric measurements.

The free radical scavenging capacity of the studied materials was investigated using the DPPH method. The best antioxidant activity was observed in the case of samples **CS-PS-I**, **PS-CS-I** and **CS2I** (Figure 4.16.), whose performance can be explained by the synergistic effect between the -OH, -NH₂ and -HC=N- functional groups present in the chemical structure of the material, as well as by the disruption of the hydrogen bond network between the hydroxyl and amino groups on the chitosan chain, thus making them available for free radical scavenging [52].

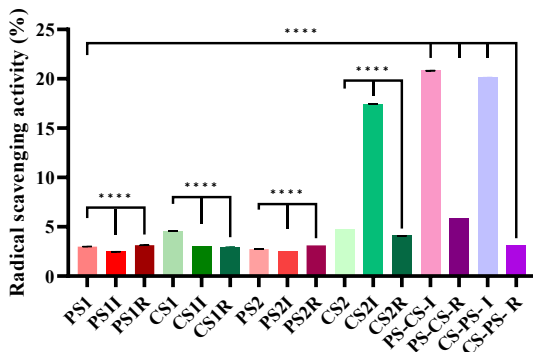


Figure 4.16. Antioxidant activity of the investigated materials in solid state

Given the ability of chitosan to induce hemostasis through direct contact with red blood cells and platelets and therefore its widespread use in wound care to stop bleeding [36], the ability of polysulfone and chitosan-based materials to promote blood coagulation was investigated, with the aim of identifying their potential application in the biomedical field. For a comprehensive analysis, the materials were compared with a commercial hemostatic sponge (gelatin-based-Surgispon) and gauze. The chitosan-based and polysulfone-based materials showed absorbance values ranging from 0.01 (CS2I) to 0.19 (CS-PS-R), much lower than the absorbance of pure untreated blood (2.1), suggesting that a large part of the blood deposited on the surface of the materials was coagulated. Moreover, most of the materials showed a coagulation efficiency superior to gauze and, in some cases (PS1, PS1I, CS2, CS2R, PS-CS-I, PS2I), even higher than that of the commercial hemostatic sponge.

Another property investigated in this study was the ability of the materials to absorb blood, with a positive impact on the coagulation process (Figure 4.20.) [37]. The highest swelling capacity was observed in the case of unmodified chitosan xerogel (11.95 mg/mg) and chitosan xerogel modified with polysulfone by amination (9.36 mg/mg). Furthermore, these materials showed the highest values of mass increase after direct contact with blood, exceeding that of the commercial hemostatic sponge (8.85 mg/mg) and that of gauze (3.18 mg/mg).

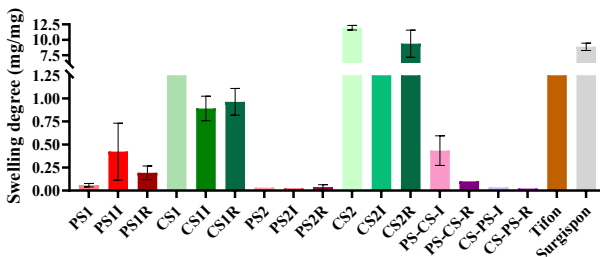


Figure 4.20. Mass swelling degree (mg/mg) of the investigated materials in blood after 10 minutes

This fact can be correlated with the very porous morphology of the material, but also with the presence of hydroxyl and amine groups in the chitosan structure that allow interaction with water molecules in blood, thus leading to blood absorption and implicitly to a significant increase in mass. It is worth noting that the modification of the chitosan xerogel did not significantly affect the porosity and hydrophilicity of the material, which suggests that the addition of the two polymers does not have a negative effect on the swelling capacity and hemostatic properties of chitosan. In contrast, the polysulfone film and xerogel, as well as the **CS-PS-I** and **CS-PS-R** samples, showed the lowest swelling capacity, with values ranging between 0.02-0.05 mg/mg. This result is similar to that obtained from the swelling experiment in PBS and can be correlated with the low affinity of polysulfone for water molecules, thus limiting their absorption.

GENERAL CONCLUSIONS

The doctoral thesis entitled “Polysulfone Materials with Biologically Active Properties” comprises 195 pages divided into five chapters, including 20 tables, 103 figures, 30 schemes, and 467 bibliographic references. The thesis is structured in two parts: a literature review (Chapter 1) and the original contributions (Chapters 2–5), concluding with the general findings drawn from the conducted studies.

The first chapter presents literature data regarding UDEL polysulfone and its derivatives, the structure–property relationship, and the main applications of polysulfone-based and polysulfone-derivative materials in the biomedical field.

The **original results**, organized into four distinct chapters, address the following research directions:

- **The synthesis and characterization of polysulfone derivatives**, including a specific study focused on evaluating their solubility through theoretical and experimental approaches, and another study focused on the use of quaternized polysulfone as a substrate for bioactive coatings;
- **The development of coatings for the treatment of fungal nail infections**, based on formylated polysulfone and amphotericin B, using the dynamic chemistry of imine bonds;
- **The synthesis and characterization of hybrid materials** based on polysulfone and chitosan linked through imine and amine bonds.

The following conclusions were drawn from the studies undertaken:

1. Two polysulfone derivatives were synthesized: polysulfone with quaternary ammonium groups (**PSFQ**) and polysulfone with sulfonic acid groups (**PSFS**). The chloromethylated derivative of polysulfone (**PSFCM**) was also synthesized, characterized, and used as an intermediate in the synthesis of **PSFQ**.

- The chloromethylation reaction protocol of PSF was optimized to obtain a derivative with a high degree of substitution, without side reactions.

- The quaternization reaction protocol of **PSFCM** was optimized to yield a **PSFQ** derivative with a reproducible substitution degree (1.35), while the sulfonation reaction protocol led to a **PSFS** derivative with a substitution degree of 0.6.
- The structures of the polysulfone derivatives were confirmed by FTIR and NMR spectroscopy (^1H -NMR, ^{13}C -NMR, ^{15}N -NMR, and two-dimensional experiments).
- Thermal characterization of the obtained polymers by TGA showed that their thermal stability decreased in the following order: PSF > PSFCM > PSFS > PSFQ.
- The solubility parameters of the functionalized polysulfones were determined using the improved group contribution method in various common solvents, and the partial solubility parameters of the synthesized polysulfones were correlated with the Hansen partial solubility parameters of the analyzed solvents.
- According to Hansen's solubility sphere theory, DMF, DMSO, NMP, and DMAc were identified as the most compatible solvents for the two ionic polysulfones.
- The solubility parameters of the polysulfone derivatives were also calculated based on intrinsic viscosity values experimentally determined for the two ionic polysulfones in DMF, DMSO, NMP, and DMAc, revealing DMF as the most thermodynamically favorable solvent.
- The most compatible solvents identified through theoretical and experimental methods were subsequently used to obtain films based on **PSFQ** and **PSFS**.
- Investigation of the topography of films cast from the synthesized polysulfone derivatives by atomic force microscopy indicated DMF as the optimal solvent for obtaining smooth films, with lower average and root-mean-square roughness values compared to films obtained using other solvents.
- The wettability of **PSFQ** and **PSFS** films was determined through contact angle measurements with different solvents, and correlation of these values with RED values obtained from theoretical calculations allowed classification of the investigated solvents into three compatibility regions.
- The hydrodynamic (reduced, specific, and intrinsic viscosities, etc.) and thermodynamic parameters of **PSFQ** and **PSFS**, as well as the interactions between macromolecular chains and solvent molecules, were investigated by viscometric measurements using the Huggins and Wolf models. These confirmed, on one hand, the polyelectrolytic nature of both polymers and, on the other hand, a greater affinity of **PSFQ** for DMF compared to **PSFS**.

2. Five formulations were obtained by combining quaternized polysulfone with an antifungal drug, amphotericin B (AmB), and/or a broad-spectrum antibiotic, norfloxacin (NFX), in different weight ratios, with the aim of creating bioactive coatings for biomedical devices.

- Structural analyses (^1H -NMR, FTIR, and UV-Vis) of the formulations demonstrated strong interactions between the polymer matrix and the two drugs.
- Morphological characterization using POM, WXR, and SEM confirmed the fine, submicrometric dispersion of the drugs within the polymer matrix.
- Surface property measurements showed water contact angle values between 60° and 90° and surface energy values in the range of 16.5–44.9 mN/m, indicating moderate wettability

and the ability to support cell adhesion and proliferation, key features for medical devices intended for contact with living tissues.

- The release kinetics of NFX showed a sustained release profile, with approximately 74 % of the drug released over 48 hours, regardless of sample composition, and a faster release of 63 ± 6.6 % within the first 4 hours.
- Monitoring the release kinetics of AmB from the polymer matrix was not possible, as the drug concentration in the supernatant fell below the UV–Vis spectrophotometer detection limit.
- Fitting the experimental NFX release data to various mathematical models suggested that NFX release from the polymer matrix is a diffusion-controlled process influenced by drug content, exposure time, and matrix hydrophilicity.
- The formulations exhibited significant antioxidant activity correlated with drug content, reaching a free radical inhibition capacity of 67.49 % for the sample with the highest drug loading, primarily attributed to amphotericin B.
- All samples demonstrated antimicrobial activity against bacterial and fungal species commonly associated with nosocomial infections: *S. aureus* (Gram-positive bacterium), *E. coli* (Gram-negative bacterium), and *C. albicans* (opportunistic microorganism), as confirmed by two *in vitro* antimicrobial assays (Japanese Industrial Standard and Kirby–Bauer), showing large inhibition zones of up to 39 mm against *E. coli* and 20 mm against *C. albicans*.
- The experimental data demonstrated the potential use of quaternized polysulfone as a matrix for encapsulating antimicrobial agents to obtain coatings for medical devices or surgical instruments capable of preventing infections at the surgical site.

3. Controlled release systems for the antifungal drug amphotericin B (AmB) were obtained through the acid-catalyzed condensation reaction of the therapeutic agent with a formylated polysulfone derivative, with the goal of applying these materials as nail polish for the topical treatment of onychomycosis.

- Structural characterization by NMR and FTIR spectroscopy confirmed the successful synthesis of the formylated polysulfone derivative.
- Imine-type polysulfone–AmB derivatives were synthesized by condensation reactions carried in different molar ratios between the aldehyde functionality of the formylated polysulfone and the amine groups of AmB. Flexible and transparent films were obtained using PEG as a viscosity-enhancing and film-forming agent.
- Structural analyses of the drug delivery systems by ^1H -NMR, FTIR, and UV–Vis spectroscopy confirmed the formation of imine bonds between the aldehyde groups of formylated polysulfone and the amine groups of AmB.
- Morphological surface and cross-section analyses by SEM revealed continuous and smooth surfaces for all samples, free of cracks, indicating the good quality of the obtained films.
- AFM microscopy revealed a rough surface topography, with average roughness values of approximately 15 nm.
- WXR data and POM images confirmed the semicrystalline nature of the obtained systems, consistent with their structural characteristics.

- Thermogravimetric analysis (TGA) indicated high thermal stability of the samples and indirectly confirmed the covalent bonding of the drug to the formylated polysulfone through the absence of a characteristic degradation step.
- Water contact angle measurements demonstrated that covalent bonding of the drug to PSF significantly improves the hydrophilicity of the material.
- The samples exhibited high hydrolytic stability, consistent with the requirements for nail polish applications, with mass loss below 10 % after one hour in aqueous medium.
- The release kinetics of AmB, monitored by UV–Vis spectroscopy, showed the materials' ability to sustain drug release, with more than 58 % released over 48 hours.
- Fitting of kinetic data to mathematical models indicated that the release of the antifungal drug from the synthesized systems is a complex process, governed equally by diffusion, swelling, and matrix erosion.
- The antifungal activity of the materials, tested by the Kirby–Bauer method on *Candida albicans*, *C. glabrata*, *C. parapsilosis*, and *Saccharomyces cerevisiae*, revealed large inhibition zones (up to 31 mm), supporting their potential application as antifungal coatings.
- Cytotoxicity assessment using the MTS assay on fibroblasts showed cell viability greater than 80 %, indicating biocompatibility and safety for bioapplications, in accordance with ISO 10993-5 standards for medical devices.

4. Hybrid materials based on chitosan and formylated polysulfone were obtained through surface imination or amination reactions, or by bulk condensation of the two polymers.

- A new formylated polysulfone derivative was synthesized *via* the reaction between chloromethylated polysulfone and *p*-hydroxybenzaldehyde, and its structure was confirmed by ¹H-NMR, ¹³C-NMR, and two-dimensional NMR experiments.
- New polysulfone–chitosan materials were synthesized through imination reactions between formylated polysulfone and chitosan using various approaches: (1) imination of the chitosan film with formylated polysulfone; (2) imination of the formylated polysulfone film with chitosan; (3) imination of chitosan xerogel with formylated polysulfone; (4) imination of formylated polysulfone xerogel with chitosan; (5) bulk imination between formylated polysulfone and chitosan. The resulting imine derivatives were subjected to reductive amination to obtain the corresponding amine polysulfone–chitosan derivatives.
- The obtained materials were structurally characterized by FTIR spectroscopy and UV–Vis spectrophotometry, both confirming the successful synthesis of the targeted derivatives.
- Wide-angle X-ray diffraction (WAXD) analysis indicated short-range layering, most likely in the form of small agglomerates, possibly resulting from hydrophobic/hydrophilic phase segregation within the materials.
- TGA analysis showed that the thermal degradation of the studied materials follows the degradation profiles of the two parent polymers, with no major changes for films but with significant shifts for bulk-synthesized materials, suggesting fine polymer mixing due to covalent bonding.
- SEM analysis revealed morphologies ranging from slightly to moderately porous, depending on the composition and synthesis method.

- Detailed AFM analysis showed generally rough surface topographies, without a clear trend in the variation of average roughness values.
- Most polysulfone–chitosan materials exhibited water contact angle values between 60° and 90°, corresponding to moderate wettability, a property known to promote cell adhesion and proliferation.
- The samples exhibited a low degree of swelling in PBS solution, attributed to both the intrinsic properties of the polymers and their covalent linkage.
- Sorption/desorption curves indicated an increase in water retention capacity of the polysulfone-based materials after modification with chitosan, from approximately 8 % to 20 %, highlighting chitosan's role in improving hydrophilicity and liquid retention capacity.
- The investigated materials exhibited good water vapor transport properties, with the best results obtained for samples in which chitosan was the dominant polymer.
- The presence of chitosan imparted enzymatic degradability to the materials, with mass losses of up to 46 %.
- The obtained systems showed mild antioxidant activity, with a free radical scavenging capacity of about 20 %, attributed to the hydroxyl and amino groups in the chitosan structure.
- The materials exhibited moderate antibacterial activity against *S. aureus* and *E.coli*.
- All synthesized systems showed a slight blood coagulation and absorption capacity, a property that supports their potential use in biomedical applications.

The original results presented in this thesis have been, or are expected to be, published as scientific articles in international ISI-indexed journals.

Publications and Papers Under Review in ISI-Indexed Journals

1. **Dumbrava O.**, Filimon A., Marin L., Tailoring properties and applications of polysulfone membranes by chemical modification: Structure-properties-applications relationship, *European Polymer Journal*, **2023**, 196, 112316, <https://doi.org/10.1016/j.eurpolymj.2023.112316>, I.F.₂₀₂₄ = 6,3.
2. **Dumbrava O.**, Rosca I., Ailincai D., Marin L., Quaternized polysulfones as matrix for the development of broad-spectrum antimicrobial coatings for medical devices, *Polymers*, **2025**, 17(13), 1869, <https://doi.org/10.3390/polym17131869>, I.F.₂₀₂₄ = 4,9.
3. **Dumbrava O.**, Ailincai D., Anisie I., Rosca I., Rusu D., Dascalu A., Stoica I., Filimon A., Marin L., Polysulfone–chitosan hybrids *via* imine chemistry: a versatile strategy for functional bioactive materials, *Materials Advances*, **2025**, 6(21), 8167-8191, <https://doi.org/10.1039/D5MA00648A>, I.F.₂₀₂₄ = 4,7.
4. **Dumbrava O.**, Filimon A., Balan-Porcarasu M., Gradinaru L., Marin L., Investigation of the structure-properties relationship of ionic polysulfones: predictive solubility analysis and solution-state characterization, *Polymer Bulletin* (sent for publication).
5. **Dumbrava O.**, Rosca I., Sandu A.I., Ailincai D., Bioactive nail coatings for antifungal therapy based on imine linkage reversibility, *Journal of Drug Delivery Science and Technology* (under review since 20th October 2025).

Papers Published in ISI-Indexed Journals (Results not included in the thesis)

1. **Dumbrava O.**, Popovici D., Vasincu, D., Popa O., Ochiuz L., Irimiciuc S.A., Agop M., Negură A., Impact of the liquid crystal order of poly(azomethine-sulfone)s on the semiconducting properties, *Polymers*, **2022**, 14, 1487, <https://doi.org/10.3390/polym14071487>, F.I.₂₀₂₄ = 4,9.
2. Filimon A., Dobos A.M., **Dumbrava O.**, Doroftei F., Lupa L., Green blends based on ionic liquids with improved performance for membrane technology: perspectives for environmental applications, *International Journal of Molecular Science*, **2022**, 23, 7961, <https://doi.org/10.3390/ijms23147961>, F.I.₂₀₂₄ = 4,9.
3. Ailincăi D., Moleavin I., Sarghi A., Fîfere A., **Dumbrava O.**, Pinteală M., Balan G.G., Rosca I., New hydrogels nanocomposites based on chitosan, 2-formylphenylboronic acid, and ZnO nanoparticles as promising disinfectants for duodenoscopes reprocessing, *Polymers*, **2023**, 12, 2669, <https://doi.org/10.3390/polym15122669>, F.I.₂₀₂₄ = 4,9.
4. Filimon A., Serbezeanu D., Onofrei M., Pelin I.M., **Dumbrava O.**, Suflet D.M., Lupa L., Development of the electrospun membranes based on quaternized polysulfones with performance and functionality in hemodialysis, *Materials & Design*, **2025**, 254, 114068, <https://doi.org/10.1016/j.matdes.2025.114068>, F.I.₂₀₂₄ = 7,9.

Scientific papers published *in extenso* in the proceeding of scientific conferences:

1. **Dumbrava O.**, Filimon A., Marin L., Impact of polysulfone functionalization with *N*, *N*-dimethylbutylamine on conformational characteristics, *Materials Today: Proceedings*, **2023**, 72, 2, 576-579, doi: 10.1016/j.matpr.2022.10.060

National projects – team member

1. PN-III-P2-2.1-PED-2019-3013, nr. 310PED/2020: *New “green” technology for advanced water treatment based on functionalized polysulfones/ionic liquids membranes*
2. PN-III-P2-2.1-PED-2021-2700, nr. 579PED/2022: *Improved technologies for the development of electrophilized polysulfonic membranes integrated in an Innovative Extracorporeal Device (EID) applicable in renal failure*

Participation at national and international conferences

a) Oral communications

1. Filimon A., **Dumbrava O.**, Dobos A.M., Onofrei M.D., *Performance of quaternized polysulfone membranes in environmental applications dictated by the ionic liquid nature*, Materials, Methods & Technologies, Burgas, Bulgaria, 19–22 August **2021**.
2. Lupa L., Tolea S.N., **Dumbrava O.**, Filimon A., *Ionic liquids-based polysulfone membranes for cadmium removal from aqueous solutions*, 11th International Conference on Environmental Engineering and Management, Muttensz, Switzerland, 8–10 September **2021**.
3. **Dumbrava O.**, Filimon A., Marin L., *Impact of polysulfone functionalization with *N,N*-dimethylbutylamine on conformational characteristics*, International Conference on

4. **Dumbrava O.**, Roșca I., Ailincăi D., Marin L., *Antimicrobial coatings based on polysulfone for medical devices*, IasiCHEM Conference 7th Edition, Iasi, Romania, 30–31 October 2025.

b) Posters

1. Filimon A., Lupa L., Serbezeanu D., Dobos A.M., **Dumbrava O.**, *Improved technologies for the development of electrospun polysulfone membranes integrated in an extracorporeal device applicable in renal failure*, 27th International Exhibition of Inventions “INVENTICA 2023”, Iasi, Romania, 21–23 June 2023.
2. **Dumbrava O.**, Filimon A., Marin L., *Theoretical and experimental approaches applied in the formulation of polysulfone based materials: Solubility parameter and intrinsic viscosity*, 21st International Balkan Workshop on Applied Physics and Materials Science-IBWAP, Constanta, Romania, 11–14 July 2023.
3. **Dumbrava O.**, Serbezeanu D., Stoica I., Bargan A., Filimon A., *New Insights in the Design of Materials Based on Polysulfones with Potential Applications in Biomedical Field: Structure–Properties Relationship*, International Conference of Physical Chemistry– ROMPHYSICHEM 17th, Bucharest, Romania, 25–27 September 2023.
4. Filimon A., Serbezeanu D., Onofrei M., **Dumbrava O.**, Rusu D., Milos E., Lupa L., *Processing of Quaternized Polysulfone/Cellulose Acetate Phthalate/Polyvinylidene Fluoride Solutions by Electrospinning to Obtain Bioactive Fibrous Membranes*, International Conference of Physical Chemistry– ROMPHYSICHEM 17th, Bucharest, Romania, 25–27 September 2023.
5. Serbezeanu D., Bubulac T.V., Anghel I., Rimbu C.M., **Dumbrava O.**, *Sustainable Packaging Applications Utilizing Organophosphorus Reinforced Poly(vinyl alcohol) Nanocomposites*, International Conference of Physical Chemistry– ROMPHYSICHEM 17th, Bucharest, Romania, 25–27 September 2023.
6. **Dumbrava O.**, Ailincăi D., Rusu D., Dascălu A., Marin L., *Polymer networks based on formylated polysulfone and chitosan: preparation and characterization*, IasiCHEM Conference 6th Edition, Iasi, Romania, 31 October–1 November 2024.
7. **Dumbrava O.**, Ailincăi D., Anisie A., Marin L., *A new approach for obtaining materials based on formylated polysulfone and chitosan linked by imine or amine units*, 5th Edition of Scientific Communications of Young Researchers - Open Door to the Future, MacroYouth 2024, Iasi, Romania, 15 November 2024.
8. Serbezeanu D., Rosca I., Peptanariu D., Aflori M., Dobos A.M., **Dumbrava O.**, Filimon A., *Performance of the polysulfonic fibrous membranes in biomedical applications: Cell-material interaction and antimicrobial activity*, International Congress “Preparing the Future by Promoting Excellence, XXXIV Edition”, Iasi, Romania, 29 February–3 March 2024.
9. Filimon A., Serbezeanu D., Peptanariu D., **Dumbrava O.**, Balan V., Milos E., Lupa L., *Quaternized polysulfones/cellulose acetate phthalate/ polyvinylidene fluoride electrospun membranes: an approach towards bioactive materials for biomedical*

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