PREPARATION OF CONTROLLED RELEASE ANTIMICROBIAL FOOD PACKAGING MATERIALS

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ABSTRACT

PREPARATION OF CONTROLLED RELEASE ANTIMICROBIAL FOOD PACKAGING MATERIALS

In this study, potassium sorbate (Psb) incorporated cellulose acetate (CA) films were developed for antimicrobial food packaging applications. The most significant characteristics of these films were their asymmetric porous structure. To achieve appropriate controlled release of Psb, the structure of the films were changed by manipulating the initial casting composition, wet casting thickness, drying temperature and number of layers. The effectiveness of the films was tested through measurement of release kinetics and antimicrobial activity on selected microorganism, *Penicillium commune*. It was found that as the initial casting composition, wet casting thickness and drying temperature increase, porosity and pore size of the films reduce leading to slower release rates. The most significant parameter affecting the release was found as the number of layers. The diffusion coefficient of Psb through multilayer films decreased by two orders of magnitude compared with single layer. Drying-induced crystallization was observed in single layer films. Higher number and larger size of crystals were observed in more porous films. Fast initial release of Psb from the single layer films which is controlled by Fickian diffusion was followed by a decreasing release rate due to slow crystal dissolution. However, in multilayer films, release rate is regulated only by diffusion of Psb through the film. All the films prepared showed growth inhibition on *P. commune.* The antimicrobial activities of single layer films were found higher than the multilayer films. The results of this study demonstrated that Psb incorporated CA films show promising potential for controlled release in antimicrobial packaging.

ÖZET

KONTROLLÜ SALIM YAPABİLEN ANTİMİKROBİYEL GIDA AMBALAJ MALZEMELERİNİN HAZIRLANMASI

Bu çalışmada, potasyum sorbat (PS) içeren selüloz asetat (SA) filmler antimikrobiyel gıda paketleme uygulamaları için geliştirilmiştir. Bu filmlerin en önemli özelliğinin asimetrik ve gözenekli yapıları olduğu belirlenmiştir. Antimikrobiyel ajanın uygun kontrollü salımını elde etmek için filmlerin yapısı film çözeltisinin başlangıç kompozisyonu, ıslak çekme kalınlığı, kurutma sıcaklığı ve film katmanı parametrelerindeki manipülasyonlarla değiştirilmiştir. Filmlerin etkinliği, aktif ajanın salınım kinetiği ve hedef organizma *Penicillium commune* üzerindeki antimikrobiyel aktivitesi ölçülerek test edilmiştir. Film çözeltisinin başlangıç kompozisyonu, ıslak çekme kalınlığı ve kurutma sıcaklığı arttıkça filmin gözenekliliği ve gözenek boyutu azalmış ve daha yavaş salım oranlarının bulunmasına neden olmuştur. İncelenen dört parametre içinde, salınımı etkileyen en önemlisinin katman sayısı olduğu bulunmuştur. Çok katlı filmlerin difüzyon katsayılarının tek katmanlara göre yüz kat daha küçük olduğu gözlenmiştir. Tek katlı filmlerde kurutma etkili kristallenme gözlenmiş ve daha gözenekli film yapılarında kristal sayısının daha yüksek ve kristal boyutunun daha büyük olduğu bulunmuştur. Tek katlı filmlerdeki Fickian difüzyonu tarafından kontrol edilen baştaki hızlı PS salımı daha sonraki aşamalarda kristallerin yavaş çözünmesinden dolayı azalmıştır. Öte yandan çok katmanlı filmlerde PS salınım oranı yalnızca Fickian difüzyonu ile kontrol edilmektedir. Hazırlanan her film seçilen organizmanın gelişimini engelleyerek organizma üzerinde antimikrobiyel etki göstermiştir. Tek katlı filmlerin çok katlı filmlere oranla daha yüksek antimikrobiyel etki gösterdiği saptanmıştır. Ayrıca, 50°C'da hazırlanan çok katmanlı film 7 günün sonunda antimikrobiyel aktivitesinin büyük bir kısmını korumuştur. Salınım ve antimikrobiyel testlerden elde edilen sonuçlar, bu çalışmada geliştirilen PS içeren SA filmlerin gida paketleme uygulamalarinda kontrollu salim ile yuksek potansiyele sahip oldugunu gostermektedir.

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A_f	Area of the film (cm^2)
C_o	Initial concentration of active agent in the film (mg/cm^3)
C_f	Concentration of active agent in the film at any time t (mg/cm^3)
C_s	Concentration of active agent in the solution (mg/ml)
D	Effective diffusion coefficient of active agent in the film (cm^2/sec)
K	Partition coefficient (cm^3 solution/cm^3 film)
L	Thickness of the film (cm)
V_s	Volume of the solution (ml)
M_t	Total amount of active agent desorbed from the film at any time t (mg)
M_∞	Total amount of active agent desorbed from the film at equilibrium (mg)
t	Time (min)
x	Position in the film (cm)
k	Crystal dissolution constant (1/min)

CHAPTER 1

INTRODUCTION

The demand for minimally processed, easily prepared and ready-to-eat 'fresh' food products is increasing everyday. This situation brings about the globalization of food trade and distribution from centralized processing. These facts result in the creation of major challenges for food safety and quality. Recently, innovative ways to inhibit microbial growth in the foods while maintaining quality, freshness, and safety have been investigated. One option is to use packaging to provide safety, quality and longer shelf life (De Roever 1998, Devlieghere, et al. 2004).

In the past, food packages were basically used to provide barrier and protective functions to protect the foodstuff against the physical and environmental damages (Han 2000). However, the growth or spoilage of microorganisms in the packaged foodstuff has still continued to cause some problems regarding the food safety and quality. In order to overcome this problem, a concept called "Active Packaging" has been proposed in which, different kinds of active substances are incorporated into the packaging material in order to improve its functionality (Han 2000). Antimicrobial food packaging is a form of active packaging which involves the usage of materials with antimicrobial properties. The most significant aim of the antimicrobial packaging is to achieve foods possessing high quality, safety and long shelf life by reducing, inhibiting or retarding the growth of microorganisms which may be present in the packed food or packaging material itself and threats the foodstuff (Appendini and Hotchkiss 2002, Choi, et al. 2005).

In traditional food packaging applications, antimicrobial agents are directly mixed into the initial food formulations in order to control microbial growth and extend shelf-life. Besides the direct mixing, the antimicrobials may also be applied to food surface by dusting, dipping or spraying (Min and Krochta 2005). Direct addition of the antimicrobial agents into the food has some disadvantages. One disadvantage is that the concentration of the directly added antimicrobial agent on the surface decreases due to its diffusion into the interior parts of the food product. Therefore, the minimum

inhibition concentration required for the inhibition of the microbial growth may not be achieved and antimicrobial compound can not selectively target the food surface (Min and Krochta 2005). Another disadvantage results from the neutralization of the added agent due to its possible complex interactions with the food components (Appendini and Hotchkiss 2002). Moreover, the direct addition of the antimicrobial agent brings about the utilization of excessive amounts of chemical additives into food materials which changes the taste of the food. Antimicrobial packaging offers an alternative way to overcome these limitations.

In antimicrobial packaging applications, different synthetic antimicrobial chemicals such as organic or inorganic acids, metals, alcohols etc. (Ozdemir and Floros 2003, Han and Floros 1998, Choi, et al. 2005) or natural biopreservatives such as enzymes or bacteriocins (Suppakul, et al. 2003, Buonocore, et al. 2003a, 2003b, 2004 and 2005, Gemili, et al. 2009) have been incorporated into various polymers. Among these antimicrobial agents, potassium sorbate is one of the most popular antimicrobial agent used in food industry. Basically it is potassium salt of the sorbic acid. It is well-known for its potential antifungal activity and generally used in the preservation of cheese, dairy products and dough (Silvia, et al. 2008). The polymeric supports commonly used for immobilizing antimicrobial agents are PVOH, cellulose acetate, cellulose triacetate, whey protein and k-carregeenan (Quintavalla and Vicini 2002, Ozdemir and Floros 2003, Buonocore, et al. 2004, Choi, et al. 2005)

The most important desired property of the antimicrobial packaging materials is the controlled release of the antimicrobial agent. A rapid release of the antimicrobial compound from the film to the food surface causes its fast consumption in a short period of time, after which the minimum concentration required for the inhibition of microbial growth is not maintained on the food surface. On the other hand, spoilage reactions on the food surface may start if the release rate of the antimicrobial agent from the film is slow. Thus, the controlled release of the active agent for a long period of time is necessary to maintain its minimum inhibitory concentration on the food surfaces.

Studies on the development of antimicrobial food packaging films with controlled release properties are increasing. Different controlled release strategies were introduced which are mainly based on changing the structure of the films. Han and Floros (1998) tried to control the release of an active agent by using a three layered film structure; first layer is the outer barrier layer, whose function is to prevent the migration of the agent to the environment. Second layer is a matrix layer which contains the active

agent, and third layer is a control layer, which controls the release of the active agent to the food. Buonocore et al. (2005) also developed multilayer films consisting of two external control layers and an inner layer containing the active agent. In another work of Buonocore et al. (2003), they tried to manipulate the release kinetics of active compounds by changing the degree of cross-link of the polymer matrix (Buonocore, et al. 2003, 2004). Ozdemir and Floros (2001) targeted to manipulate the release by changing the degree of plasticization in the polymer film. Guzey and McClements (2006) proposed micro and nano-encapsulation of food ingredients as another alternative method to control the release rate of the active agent. They suggested that the release rate of the active agent from small capsules directly added into the food can be controlled by changes in pH, temperature or ionic strength of the medium. Recently, Gemili et al. (2009) introduced the usage of porous asymmetric films for food packaging applications. They tried to contol the release rates by changing the degree of asymmetry and porosity of the films.

The objective of this study is to develop potassium sorbate (Psb) incorporated asymmetric and porous cellulose acetate (CA) films for food packaging applications and investigate their efficiency in controlling the release rate of Psb. To achieve this task the cellulose acetate films with different morphological characteristics including fixed amount of Psb were prepared by changing the initial casting composition, drying temperature, wet casting thickness and number of layers. The release rates of Psb from these films were determined and the antimicrobial activity of the films was tested on selected microorganism, *Penicillium Commune.*

This thesis consists of six sections. After giving a brief introduction in the first part, in the second part, antimicrobial food packaging systems are discussed. Models used for determining antimicrobial agent transport in food packaging systems are reviewed in the third section. Materials and methods used in this study were given in the fourth section. In the fifth section, the results are shown and discussed and finally in the sixth section conclusions are given.

CHAPTER 2

ANTIMICROBIAL FOOD PACKAGING

As it is known, growth of bacteria in the foodstuff causes many undesirable effects such as life-threatening toxins, changes in the odor, color, taste and texture of food etc. These kinds of changes lead to reduction in the shelf life of the product which decreases the safety of food and treats the security of public health (Han 2005).

In the past, food packaging materials were generally used to provide only barrier and protective function (Han 2000). These traditional functions include gas barrier, protection against the transmission of oxygen and water vapor etc. However, today, by using a concept called “Active Packaging”, different kinds of active substances can be incorporated into the packaging material so that its functionality can be improved and new functions can be provided (Han 2000). These additional functions provided by active packaging technology, enable the package to interact with the food so that food quality and safety may be improved (LaCoste, et al. 2005). Among many active packaging applications such as moisture-control packaging, oxygen and carbon dioxide scavengers, polymer films with selective gas permeability, and microwave susceptors, antimicrobial packaging is one of the most promising innovations of active packaging technologies (Han 2005).

Antimicrobial packaging has an important effect on food packaging when the shelf-life extension and food safety of food products are taken into consideration. The basic idea behind this technology is the usage of antimicrobial substances in polymeric matrices, which helps the control of the microbial population and target specific microorganisms, in order to achieve higher safety and quality food products with extended shelf life. Various types of antimicrobial substances such as organic acids and their salts, enzymes, bacteriocins, and miscellaneous compounds like triclosan, silver, and fungicides have been used in synthetic polymers and edible films (Quintavalla and Vicini 2002). Different types of antimicrobial food packaging systems can be constructed by using antimicrobial packaging materials and/or antimicrobial agents inside the package space or inside foods (Han 2000, 2005).

2.1. Types of Antimicrobial Food Packaging Systems

The main phenomenon in active packaging systems is the incorporation of antimicrobial substances in food packaging materials. The antimicrobial compound incorporated into food packaging material, which is generally a polymer, is embedded in the polymeric matrix and act when the package is brought in contact with a moist food or a liquid-like food. The preservative, which is embedded into the matrix in the dry state, is released from the material and acts directly into the food.

Most food packaging systems represent either a package/food system or a package/headspace/food system.

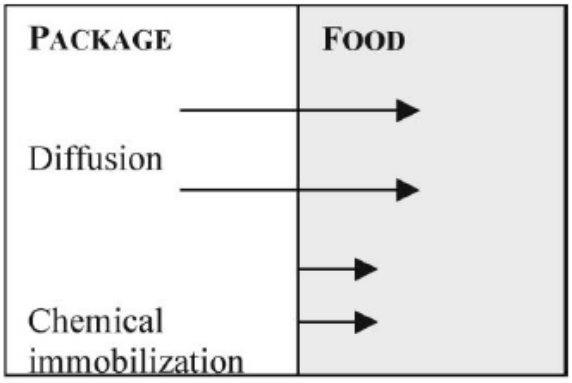


Figure 2.1. Package/Food system and relative behavior of active substances. (Source: Han 2000)

In a package/food system, as shown in Figure 2.1, solid or liquid food product is in direct contact with the packaging material. In this system, the main migration phenomena involves the diffusion between the packaging material and the food and partitioning at the interface. Hence, antimicrobial agents incorporated into the packaging materials migrate into the food through diffusion and partitioning (Quintavalla and Vicini 2002).

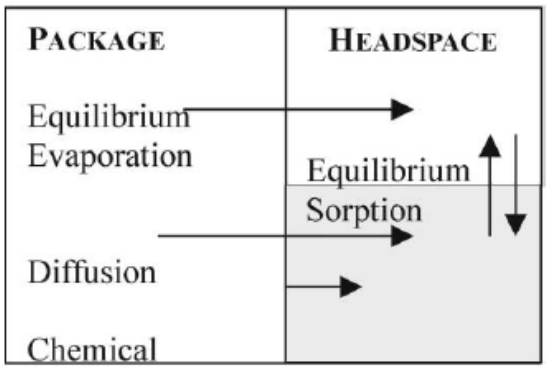


Figure 2.2. Package/headspace/food system and relative behavior of active substances. (Source: Han 2000)

Package/headspace/food systems are represented in Figure 2.2. Different from the package/food system, the main migration phenomena in this system is the evaporation of a substance to the headspace, therefore, a volatile active substance should be used. Then, the equilibrated amount of active substance may diffuse into the food (Quintavalla and Vicini 2002).

In addition to the diffusion and equilibrated sorption, covalently immobilized antibiotics, fungicides, or active moieties are used in the antimicrobial packaging applications. In this situation, the surface inhibition of microbial growth is obtained by immobilization of the non-food grade antimicrobial substance in which the diffusional mass transfer does not occur (Appendini and Hotchkiss 1997, Quintavalla and Vicini 2002).

The forms of antimicrobial food packaging systems can be listed as follows:

1. Addition of sachets-pads containing volatile antimicrobial agents into packages

Oxygen absorbers, moisture absorbers and ethanol vapor generators are the main types of sachets. Although oxygen and moisture absorbers are not antimicrobial agents, they indirectly inhibit microbial growth. For instance, reduction of headspace oxygen in the package inhibits the growth of aerobes. Also, moisture absorbers reduce water activity and indirectly affect microbial growth on the food (Appendini and Hotchkiss 2002).

2. Incorporation of volatile and non-volatile antimicrobial agents directly into polymers

There are two ways of incorporation of antimicrobials into the packaging materials. One way is the addition of antimicrobial agents into the melt form of polymer and the other is the addition into the wet polymer solution. Packaging materials should be in contact with the surface of the food for the diffusion of the non-volatile agent to the surface of the food (Ouattara, et al. 2000, Buonocore, et al. 2003, Choi, et al. 2005, Mecitoglu, et al. 2006). For the volatile antimicrobial agents such as chlorine dioxide, sulfur dioxide, carbon dioxide etc. packaging materials do not need to be in contact with the surface of the food (Appendini and Hotchkiss 2002, Suppakul, et al. 2003).

3. Coating or adsorbing antimicrobials onto polymer surfaces

This technique is generally used for the antimicrobials which are sensitive to high temperature, such as enzymes, and can not be used in polymer processing. Because of this reason, they are often coated onto the material or added to cast films (Guillard, et al. 2008).

4. Immobilization of antimicrobials to polymers by ion or covalent linkages

If both antimicrobial agent and the polymer have functional groups, immobilization of the antimicrobial agents to polymers by ionic or covalent bonding occurs. Antimicrobials with functional groups are peptides, enzymes, polyamines organic acids etc. and polymers used for food packaging applications that have functional groups are ethylene vinyl acetate (EVA), ethylene methyl acrylate (EMA), ionomer, nylon and polystyrene (PS) etc. In addition to functional antimicrobials and polymer supports, immobilization may require the use of ‘spacer’ molecules that link the polymer surface to the bioactive agent (Conte, et al. 2007).

5. Use of polymers that are inherently antimicrobial

Cationic polymers such as chitosan and poly-L-lysine are inherently antimicrobial and have been used in films and coatings. These polymers interact with

negative charges on the cell membrane and cause the leakage of their intracellular components (Appendini and Hotchkiss 2002).

2.2. Types of Antimicrobial Agents

There are different microorganisms such as molds, yeasts, bacteria etc. degrading and deteriorating the foodstuff and reducing their quality and shelf life. Most of these organisms may be inhibited by using different antimicrobial agents. These antimicrobial agents may be used either alone or in combination with each other. Moreover, these antimicrobial agents may also be used in combination within a polymeric matrix in order to achieve more efficient antimicrobial properties. Antimicrobial agent can be described as a chemical or natural compound that either destroys or inhibit the growth of microscopic and submicroscopic organisms. Antimicrobial agents can be divided into two categories according to their sources as chemical antimicrobial agents and natural antimicrobial agents. (Appendini and Hotchkiss 2002).

2.2.1. Natural Antimicrobial Agents

Nowadays, the consumption of the natural preservative containing foods is becoming popular when the health concerns are taken into consideration. As a result of this, the usage of natural antimicrobial agents in foods and food packaging materials has gained a major importance. The most popular antimicrobial agents are enzymes such as nisin, lysozyme, lactoperoxidase, etc.

Nisin is an antibacterial polypeptide produced by *Lactococcus lactis* subspecies lactis that broadly inhibits gram-positive bacteria and spore formers (Pedgett, et al. 1998) but when combined with a chelator, nisin also can inhibit growth of some gram-negative bacteria (Dawson, et al. 2005).

Lysozyme, found in different sources including plants, animals and microorganisms, is a single peptide enzyme (Ibrahim, et al. 1996). Its lytic activity on bacteria occurrs by hydrolyzing glycosidic linkages between N-acetylhexosamines of pepdidolycan in their cell walls. The antimicrobial activity of the enzyme is mainly against gram-positive bacteria but because of the peptidoglycan layer surrounded by a protective lipopolysaccharide membrane, it is ineffective against gram-negative bacteria (Nakamura, et al. 1991, Ibrahim, et al. 1991). However, its usage in combination with EDTA makes it efficient against gram-negative bacteria (Mecitoglu, et al. 2006, Gemili, et al. 2009).

2.2.2. Chemical Antimicrobial Agents

A chemical which has an ability to prevent or delay deterioration, may be considered as chemical antimicrobial agent.

Benzoic acid is used as a chemical antimicrobial agent in food industry. It occurs in pure form as colorless or white needles or leaflets and soluble in water (Davidson and Branen 1993).

Sorbic acid is another chemical antimicrobial agent which is widely used in recent years. Sorbic acid and its water soluble salts, especially potassium sorbate, are used as preservatives for various foods, animal feeds, pharmaceuticals, cosmetics and in other applications. They inhibit or delay the growth of many microorganisms, including yeasts, molds, and bacteria (Davidson and Branen 1993).

Potassium sorbate is the potassium salt of sorbic acid. It is prepared by the reaction of sorbic acid with potassium hydroxide. Potassium sorbate is effective in a variety of applications including food, wine, and personal care. However, its primary use is as a food preservative.

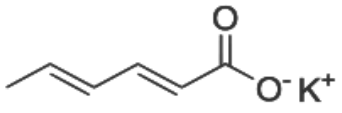


Figure 2.3. Molecular structure of potassium sorbate.
(Source: Wikipedia 2009)

The molecular formula of potassium sorbate is $C_6H_7O_2K$ and its systematic name is potassium (E,E)-hexa-2,4-dienoate. It has a molecular weight of 150.22 g/mol and highly soluble in water (58.2% at 20 °C) (Wikipedia 2009).

Potassium sorbate is used to inhibit molds and yeasts in many foods, such as cheese, wine, yogurt, dried meats, apple cider and baked goods. It can also be found in the ingredients list of many dried fruit products. In addition, herbal dietary supplement products generally contain potassium sorbate, which acts to prevent mold and microbes and to increase shelf life, and is used in quantities at which there are no known adverse health effects. Also, it is used in many personal care products to inhibit the development of microorganisms for shelf stability (Wikipedia 2009).

Potassium sorbate is considered to be safe because of its long term safety record and non-toxic profile. Potassium sorbate is non-irritating and non-sensitizing. Allergic reactions are rare and it is well tolerated when administered internally. The lethal dose for potassium sorbate is 4.92g/kg or 344g for a normal adult. The amount of potassium sorbate in a serving (100g) is typically between 0.025% to 0.1% or 25mg to 100mg, which is 0.1% to 0.3% of the lethal dose. Acceptable daily intakes for human are 12.5mg/kg, or 875mg daily for a normal adult according to FAO/World Health Organization Expert Committee on Food Additives (Wikipedia 2009).

Common food processing and storage conditions do not affect the stability of sorbates significantly. However, heat processes can reduce the antimicrobial activity of potassium sorbate. It was found that potassium sorbate lost its antimicrobial activity exponentially with temperature and linearly with heating time (Han and Floros 1998). This loss could be very serious because insufficient antimicrobial activity may lead to microbial spore germination. Potassium sorbate has two double bonds in its structure as shown in Figure 2.3. Because of this, its color may change due to oxidative degradation of the double bonds. Other food components such as minerals, carbohydrates and amino acids may also accelerate the oxidation process. Generally, the decomposition of sorbate is accelerated by decreasing pH, sodium chloride, potassium chloride, sugar and metal concentrations and increasing acetic acid, glycerol, majority of salts and amino acid concentrations.

The color of potassium sorbate turns dark yellow upon heating. The color change of potassium sorbate is initially caused by oxidation of the double bonds of the sorbate chain. Thus, the final color of a food product containing potassium sorbate as a preservative could also become darker and yellow after thermal processing (Han and

Floros 1998). Its maximum concentration in foods is regulated at 0.2 – 0.3 % (Wikipedia 2009). Potassium sorbate has a tendency to be crystallized by solvent evaporation and effect of temperature since it is the potassium salt of the sorbic acid.

2.3. Previous Studies

2.3.1. Previous Studies on Classical Antimicrobial Food Packaging Systems

There are many studies related with the preparation of different antimicrobial food packaging systems. For instance, Hansen et al. (1989) incorporated the organic acids and surfactants into absorbing pads, which are used in trays for packaged retail meats and poultry to soak up meat exudates, to prevent microbial growth in the exudates, which are rich in nutrients.

Thermal processing techniques such as extrusion and injection molding were used to incorporate thermally stable antimicrobials such as zeolites into the polymers. Using these techniques, Brody et al. (2001) added silver exchanged zeolites into polymers such as polyethylene, polypropylene, nylon and butadiene styrene at levels of 1-3%. For heat-sensitive antimicrobials like enzymes and volatile compounds, solvent compounding was used for their incorporation into polymers. For instance, Appendini and Hotchkiss (1997) incorporated lysozyme into cellulose ester films by solvent compounding in order to prevent heat denaturation of the enzyme.

Cast edible films, have been used as carriers for antimicrobials and applied as coatings onto packaging materials and foods. For example, in the study of Cooksey et al. (2000), the nisin/methylcellulose coatings for polyethylene films and nisin/zein coatings for poultry were investigated. Bower et al. (1995) demonstrated that nisin adsorbed onto silanized silica surfaces inhibited the growth of *L. monocytogenes*. A similar study of them showed that surfaces with low hydrophobicity had more nisin activity than those with higher hydrophobicity, even if adsorbed mass values were generally the inverse. Other examples include the adsorption of nisin on PE, EVA, PP,

polyamide, PET, acrylics and PVC. Ming et al. (1997) studied the pediocin-containing milk-based powders adsorbed onto cellulose casings and barrier bags. Natrajan and Sheldon (2000) investigated the nisin/EDTA/citric solutions coated onto PVC, nylon and LLDPE films. Poly(ethylene-co-methacrylic acid) films treated with sodium hydroxide and swollen with acetone showed an increased absorption and diffusion of benzoic and sorbic acids compared to non-treated films (Weng and Chen 1999). It was found that these NaOH treated films had the highest inhibitory effect on molds. They claimed that this was due to the fact that the higher polarity of NaOH treated films enhanced the absorption of the antimicrobials. An et al. (2000) studied the usage of binders, such as polyamide resins, for the increment of the compatibility between polyolefins surfaces and bacteriocins. Labuza and Breene (1989) investigated the glucose oxidase coated onto moisture proof fabric sheets by using polyvinyl alcohol, starch and casein as adhesives.

Immobilization of antimicrobials by ionic or covalent linkages to polymers is another system used in food packaging. For example, Soares and Hotchkiss (1998) used the substrate to protect and increase the activity of naringinase immobilized in cellulose acetate films. Appendini and Hotchkiss (1997) used the covalently immobilized forms of lysozyme and chitinase against gram positive bacteria. Garcia and Galindo (1990) managed to covalently bind glucose oxidase enzyme onto insoluble supports that could be compatible with packaging materials. Garibay et al. (1995) co-immobilized the beta-galactosidase and glucose oxidase in order to produce hydrogen peroxide to activate lactoperoxidase in milk.

Moreover, the usage of polymers that are inherently antimicrobial may be considered as another system. Goldberg et al. (1990) demonstrated that cationic polymers such as chitosan and poly-L-lysine promote cell adhesion, since charged amines interact with negative charges on the cell membrane, causing leakage of intracellular constituents. Chitosan has been used as a coating and appears to protect fresh vegetables and fruits from fungal degradation (Appendini and Hotchkiss 2002). In addition, Ouattara et al. (2000) studied the chitosan based antimicrobial films to carry organic acids and spices. Calcium alginate films reduced the growth of the natural flora and coliform inocula on beef, possibly due to the presence of calcium chloride (Cuq, et al. 1995). Pardini (1987) proposed the bactericidal acrylic polymers made by co-polymerizing acrylic protonated amine co-monomer as packaging materials for

increased fruit and vegetable shelf life. Olstein (1992) indicated that polymers containing biguanide substituents also yield antimicrobial activity.

Sorbates are very important antimicrobial agents and there are many studies related with their usage in the food packaging applications. For instance, Vojdani and Torres (1989) found that sorbates are rapidly absorbed from food surfaces, loosing the protective effect. They incorporated sorbates into polysaccharide films and demonstrated that the films allowed slower diffusion of the sorbates to the surface of a food, which in turn improved surface protection. Films with low diffusion rates were desirable since they maintained higher surface concentrations of sorbate for longer periods. Pectin/gluten/monoglyceride films containing sorbic acid have also been shown to delay the growth of molds in model food systems, as compared to sorbic acid deposited directly into the food's surface by Guilbert et al. (1997). Torres and Karel (1985) carried out extensive studies focusing on sorbic acids and its salts incorporated into zein films and mixtures of fatty acids and cellulose derivatives. These films combined with low surface pH have been shown to improve microbial stability in food model. Other early developments included coating wax paper and cellulose casings with sorbic acid for wrapping sausages and cheeses (Labuza and Breene 1989). Choi et al. (2005) incorporated the potassium sorbate into k-carrageenan while Ozdemir and Floros (2001) added it into edible whey protein films.

2.3.2. Previous Studies on Controlled Release Food Packaging Systems

Controlled release packaging, which is a challenging area in the active food packaging, is a fairly new concept and can be used to enhance the quality and safety of a wide range of foods and extend their shelf life (LaCoste, et al. 2005). Here the main idea is to use the package as a delivery system for active compounds, such as antimicrobials, antioxidants, enzymes, flavors and nutraceuticals, and provide the release of the active agent in a controlled manner so that the continuous and target specific microbial inhibition is achieved.

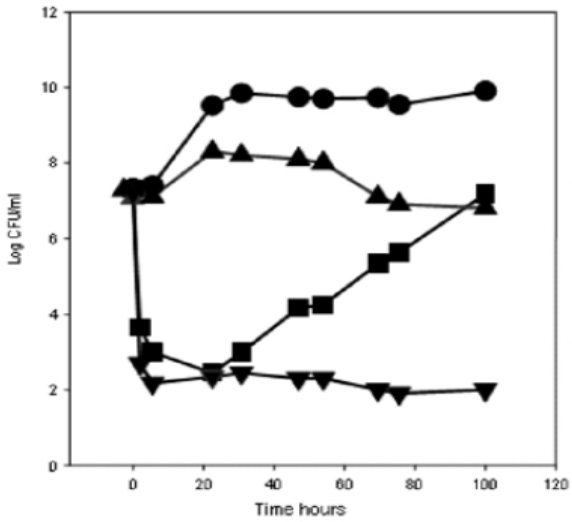


Figure 2.4. Different delivery modes of nisin. Instant addition of nisin (■). Slow addition of nisin (▲). A combination of the two delivery modes mentioned (▼).The control free of nisin (●). (Source: LaCoste, et al. 2005)

Figure 2.4 shows the importance of controlled release for a model active compound nisin. The instant addition of nisin results in almost immediate kill of microorganisms and its fast consumption. On the other hand, very slow addition of the same amount of nisin does not cause any dramatic reduction in microbial growth. However, when the combined delivery mode is applied, which corresponds to the controlled release, it provides the most effective microbial inhibition over time, indicating that slow addition can work synergistically with instant addition. These results obviously depict the efficiency of controlled release on the inhibition of microorganisms and thereby, improvement of food quality and safety (LaCoste, et al. 2005).

Different methods were proposed in order to control the release rate of the active agent from polymeric films for food packaging applications. Buonocore et al. (2003a) entrapped lysozyme into a highly hydrophilic dry polymer PVOH and crosslinked the matrix by using glyoxal as a crosslinking agent. They tried to regulate the release rate of lysozyme by changing the amount of crosslinking agent added into the polymer. Their measurements indicated that with the increased degree of crosslinking in the film, the amount of water sorbed at equilibrium, hence, the diffusion coefficient of lysozyme decreased. In addition, the amount of lysozyme entrapped in the film at equilibrium increased. In their work, a mathematical model was also derived which takes into account diffusion of water molecules into the polymeric film and the counter - diffusion

of the incorporated antimicrobial agent from the film into the aqueous solution. Based on experimental observations and model predictions, they explained the release kinetics with the diffusion of water into the matrix, the relaxation of polymer chains and the diffusion of the active compound through the swollen polymer network.

In another work, Buonocore et al. (2005) prepared PVOH based multilayer films which consist of control layer, active matrix layer including lysozyme as an antimicrobial agent and barrier layer. The composition of each monolayer and multilayer films is shown in Table 2.1.

Table 2.1. The compositions of crosslinking agent in different films.
(Source: Buonocore, et al. 2005)

Sample	**Monolayer**	**Multilayer**		
	Containing Lysozyme	**Layer 1**	**Layer 2 Containing Lysozyme**	**Layer 3**
Film A	Cross-link A*			
Film B		Cross-link A	No cross-link	Cross-link A
Film C		Cross-link A	Cross-link A	Cross-link A
Film D	Cross-link B**			

* : 0.077% (w/w) of glyoxal
**: 7.7% (w/w) of glyoxal

They observed that Film A, which posses a lower crosslinking degree than that of Film D, has faster release kinetics and higher amount of lysozyme at equilibrium. Secondly, they made a comparison between the behaviour of monolayer film A and multilayer film C, which consists of cross-linked PVOH film (Film A) in its first and third layers as barrier and control layer. They detected a slow diffusion and a lower amount of lysozyme at equilibrium from Film C as compared to Film A. They explained this situation with additional mass transfer resistance to the diffusion of lysozyme through the matrix due to presence of inner and outer layers which resulted in slow release kinetic and a decrease in the amount of lysozyme released at equilibrium with respect to Film A.

Finally, they compared the release behavior of two multilayer Films B and C. While Film B was containing a non cross-linked PVOH layer as central layer, Film C

was containing a crosslinked central layer. For both Film B and C, the first and third layers had the same crosslinking degrees. Experimental data showed that a higher equilibrium quantity of lysozyme is released from Film B with respect to Film C. In the Film B, since the glyoxal was not present in the inner layer, the enzyme is not chemically bonded to the PVOH backbone. It was basically entrapped in the meshes of polymeric network. So, here the release rate was controlled only by the control layer. However, in Film C, in addition to the control layer, there was a cross-linking between the lysozyme and the polymeric backbone in the central layer which also controls the release of the agent.

Ozdemir and Floros (2001), investigated the mechanism of potassium sorbate release from edible whey protein films. A model describing the diffusion of potassium sorbate from whey protein films that swelled due to countercurrent diffusion of solvent was used to determine potassium sorbate and solvent diffusion coefficients. In the film preparation, whey protein isolate (WPI) was used as polymeric matrix, sorbitol as plasticizer and potassium sorbate as antimicrobial agent. They also investigated the effect of degree of plasticization on the release rate of potassium sorbate. It was found that the films prepared with higher plasticizer concentration have faster potassium sorbate release and higher diffusion coefficient values. They reported that increased initial potassium sorbate concentration in the films results in an increase in diffusion coefficients due to the high water solubility of potassium sorbate, which increased the hydrophilicity of the polymer matrix and facilitated solvent absorption when in contact with the glycerol/water solution. Their results indicated that the diffusion coefficients of potassium sorbate in edible whey protein films were approximately one order of magnitude smaller than those determined in intermediate moisture food systems. Thus, they claimed that whey protein films can be used to carry and deliver antimicrobial substances such as potassium sorbate.

Gemili et al. (2009) investigated the controlled release of lysozyme, produced from hen egg white, from cellulose acetate films. They obtained antimicrobial films by incorporation of lysozyme into cellulose acetate (CA) films which possess asymmetric porous structure. They controlled the release of lysozyme by changing the structure of films from highly asymmetric and porous to dense. This was achieved by increasing the concentration of CA from 5% to 15% in the initial casting solution. The highest release rate, soluble lysozyme activity and antimicrobial activity were obtained for the film prepared from 5% CA solution including 1.5% lysozyme. Due to asymmetric nature of

the films, which results in different morphologies on each surfaces, they reported that the release rate of lysozyme is lower from the dense surface as compared to the porous surface of the film.

2.4. Antimicrobial Tests

2.4.1. Antimicrobials and Target Microorganisms

The microorganisms and their inhibiting antimicrobial agents are shown in the following table.

Table 2.2. Microorganisms and their inhibiting antimicrobial agents (Source: Appendini and Hotchkiss 2002)

Antimicrobials	Polymer/carrier	Main target microorganisms
Organic acids / anhydrides: Propionic, benzoic, sorbic, acetic, lactic, malic	Edible films, EVA, LLDPE	Molds
Inorganic gases: Sulfur dioxide, chlorine dioxide	Various polyolefins	Molds, Bacteria, Yeasts
Metals: Silver	Various polyolefins	Bacteria
Fungicide: Benomyl, imazalil	LDPE	Molds
Bacteriocins: Nisin, pediocins, lacticin	Edible films, cellulose, LDPE	Gram-positive bacteria
Enzymes: Lysozyme, glucose oxidase	Cellulose acetate, PS	Gram-positive bacteria
	Edible films	
Chelating agents: EDTA	Edible films	Gram-negative bacteria
Spices: Cinnamic, caffeic, *p*-coumaic acids	Nylon/PE, cellulose	Molds, yeast, bacteria
Horseradish (allylisothiocyanate)		
Essential oils (plant extracts): Grapefruit seed extract, hinokitiol, bamboo powder, Rheum palmatum, Coptis chinesis extracts	LDPE, cellulose	Molds, yeast and bacteria
Parabens: Propylparaben, ethylparaben	Clay-coated cellulose	Molds
	LDPE	
Miscellaneous: Hexamethyl-enetetramine	LDPE	Yeasts, anaerobes and aerobes

Abbreviations: EVA (ethylene vinyl acetate); LLDPE (linear low density polyethylene); LDPE (low density polyethylene); PS (polystyrene); PE (polyethylene).

The determination of the efficiency of the antimicrobial agents against the target microorganisms is a critical concept for the quality of the foods. The more an antimicrobial agent is efficient on a selected microorganism, the more the quality and shelf life of food is obtained. There are different methods used to determine the efficiency of the antimicrobial agents against the target organisms. (Appendini and Hotchkiss 2002).

2.4.2. Antimicrobial Test Methods

Agar plate methods, determination of minimum inhibitory concentrations (MIC) and dynamic shake flask tests are some of the methods used to determine the antimicrobial efficiency of the antimicrobial agents used alone or in combination with a polymer.

Basically MIC may be described as the lowest concentration of an antimicrobial in a polymer resulting in the complete inhibition of growth of a test microorganism. In the MIC method, a series of tubes containing growth medium with the target microorganism and with polymers containing different concentrations of antimicrobial are seeded. The tubes are then incubated for a predetermined period of time and visually inspected for microbial growth (Appendini and Hotchkiss 2002).

In the agar plate test, antimicrobial film is placed on a solid agar medium containing the test microorganism. The agar plates are incubated until growth is visible. A clear zone surrounding the film indicates antimicrobial diffusion from the film and subsequent growth inhibition. Lack of growth under a film may indicate inhibition, but appropriate controls must be included. The agar plate test method simulates wrapping of foods and may suggest what can happen when films in contact with contaminated surfaces and the antimicrobial agent migrates from the film to the food. The method can be quantitative if the diameter of the clear zones around the films is measured (Appendini and Hotchkiss 2002).

Shake flasks tests provide more detailed information on antimicrobial kinetics. Liquid media buffer, growth media or foods are seeded with the target microorganisms and the antimicrobial polymer. The flasks are incubated with mild agitation. Samples

are taken over time and enumerated. Unlike the MIC test, this method measure reduction in growth rate even if substantial grow occurs. Tests in buffer provide information on the microbial properties of the polymers while tests in broth provide information on microbial growth kinetics and the antimicrobial mode of action of the polymers. Tests in buffer may be misleading since cells susceptible in nutrient-poor media may recover if nutrients are present. When testing antimicrobial films by the shake flask test, the ratio of film surface area to volume of product or media must be considered. Increasing the surface area/volume ratio increases the activity of bioactive molecules incorporated into polymer films. From an antimicrobial standpoint, high surface/volume ratios may seem adequate. But in real packaging applications, surface area/volume ratios of one are considered optimal, and values higher than that may be impractical. By accounting the area/volume ratio, the feasibility of such films for practical applications may be assessed. As its name implies, the shake flask test includes agitation, which enhances the contact between the antimicrobial polymer and the cells. (Appendini and Hotchkiss 2002)

2.5. Previous Studies on Antimicrobial Tests

In the literature, there are numerous studies related with the antimicrobial efficiency of various films against different microorganisms. Here, only a few studies will be reviewed on the antimicrobial efficiency of potassium sorbate or on the inhibition of *P. commune* which are used as model antimicrobial agent and microorganism in this study.

Min and Krochta (2005) studied the effects of lactoferrin (LF), lactoferrin hydrolysate (LFH), and lactoperoxidase systems (LPOS), both directly and incorporated into edible whey protein isolate (WPI) films, on the inhibition of *P. commune*. They examined the antimicrobial effects by turbidity, disc diameter, surface spreading, and film surface inoculation tests. The compositions of LPOS were changed as shown in Table 2.3 in order to study its effect on the growth of *P. commune.*

Table 2.3. Composition of lactoperoxidase systems.
(Source: Min and Krochta 2005)

System nr	Lacto-peroxidase (LPO, 2.3 mg)	Glucose-oxidase (GO, 0.8 mg)	Glucose (Glu, 250 mg)	KSCN (2.5 mg)	KI (4 mg)	H_2O_2 (5 mL)	Butterfield's buffer (250 mg)
1					•	•	
2	•			•	•	•	•
3	•				•	•	•
4	•			•		•	•
5	•	•	•	•	•	•	
6	•	•	•	•	•		
7	•	•	•		•		
8	•	•	•	•			

[a]The system only with H_2O_2 and Butterfield's buffer.
[b]Blank, indication of absence; •, indication of presence.

In turbidity tests, the changes in the absorbance of 1% peptone water and potato dextrose broth (PDB) containing different concentrations of LF, LFH, and LPOS after inoculation of *P. commune* were investigated. They observed the inhibition of *P. commune* in 1% peptone water for 2 days and 5 days with LF higher than 0.5 mg/ml and 10 mg/ml, respectively. The MIC of LF was determined as 10 mg/ml for the inhibition of *P. commune* from $3.6x10^3$ spores/ml in 1% peptone water (50 µl of $3.6x10^5$ spores/ml in 5 ml 1% (w/w) peptone water). *P. commune* was found to be more resistant to LF than those bacteria that were investigated by other researchers in 1% peptone water. The results indicated no inhibition effect of LF in PDB medium. They claimed that this may be due to high concentration of divalent cations, including ferric ions, in nutrient-rich PDB. They implied that LF looses its antimicrobial activity when it is saturated with divalent cations including ferric ions. This result was consistent with the results of the other researchers that also reported a reduction in inhibitory activity of LF in divalent cation rich media.

In the second case, they observed the inhibition with LFH at concentrations higher than 10 mg/ml in 1% peptone water, indicating a MIC of 10 mg/ml for LFH in 1% peptone water with inoculum size of $3.6x10^3$ spores/ml. They found that *P. commune* used in their study was more resistant to LFH than those bacteria used in other researches. However, they did not come across any inhibition with LFH in PDB. They argued that this is because LFH contains a potent LF-derived antimicrobial peptide named lactoferricin that has higher antimicrobial activity than LF. However, similar to LF, antimicrobial effect of LFH depended on the amount of cations.

In disc diameter tests, they found that films incorporating 0.1 g/g (dry basis) LF or LFH did not produce any zones of inhibition of the growth of *P. commune* from 10^3 to 10^5 spores on dichloran rosebengal chloramphenicol (DRBC) agar. This was

attributed to the presence of divalent cations in the LOPS. The results from the disc diameter test indicated that the *P. commune* growth was sensitive to the films including the LPOS, and an efficient inhibition was obtained with System 5 shown in Table 2.3. However, they did not observe any inhibition with Systems 2, 6, and 8, even though these systems exhibited effectiveness in the turbidity test. They thought that this might be due to the high efficiency of System 5 in generating oxidizing products such as hypothiocyanite and hypoiodite, which inhibit the growth of *P. commune*. They saw the clear zones of inhibition on DRBC agar with the films denoted as System 5, including 59 mg/g LPO. The inhibition effect with this film was not obscured when the number of spores was 10^5.

The results from this research indicated that LF and LFH with concentration higher than 10 mg/ml were effective against *P. commune* in 1% peptone water but not in PDB. LF and LFH were also not effective against *P. commune* when incorporated into WPI films. LPOS with concentration higher than 0.1% (w/w) inhibited *P. commune*, in both 1% peptone water and PDB. Furthermore, LPOS incorporated into WPI films at a level of 59 mg LPOS/g film inhibited growth of *P. commune*. Thus, they concluded that WPI films with LPOS have potential to be used with complex food systems, for which LF and LFH may have limited effectiveness.

Silvia et al. (2008) developed new hydroxypropyl methylcellulose (HPMC)-lipid edible composite films containing low-toxicity chemicals such as salts of organic acids, salts of parabens, and mineral salts etc. with antifungal properties and they investigated the antifungal activity of selected films containing food preservatives for *in vitro* evaluation against *Penicillium digitatum* (PD) and *Penicillium italicum* (PI). They evaluated the antifungal activity of selected edible films through the disc diameter test for three levels of inoculum prepared for each fungal species: 10^3, 10^4, and 10^5 spores/mL. They used potato dextrose agar (PDA) and dichloran rose-bengal chloramphenicol agar (DRBC) as the media for the disc diameter test.

They found that among all organic acid salts added into HPMC-lipid films, only films containing potassium sorbate (PS) (pH 7.42) or sodium benzoate (SB) (pH 7.33) clearly inhibited ($p < 0.05$) the growth of both PD and PI at 10^3-10^5 spores/ml on DRBC agar. It was proved that films with PS or SB better controlled PD than PI. According to their results, films containing 2.0% PS produced larger inhibition zones for PD and PI than films with 2.5% SB. In general, it was observed that films with other organic acid

salts did not inhibit both PD and PI. The only exceptions were SP, sodium formate, and sodium citrate that inhibited PD at 10^3 spores/ml.

Moreover, in this study, they tried to use mixtures of the most effective organic acid salts in HPMC-lipid films for the goal of achieving an additive or synergistic effect for the inhibition of PD or PI. According to them, films with a mixture of PS + SP significantly inhibited ($p < 0.05$) the growth of PD on DRBC agar at all inoculum concentrations tested. They found that PD at 10^3 spores/ml was more intensely inhibited than at 10^4 or 10^5 spores/ml. Therefore, they claimed that the inhibition ability was greatly dependent upon the pathogenic inoculum density. In contrast, they observed that PI was not inhibited at all by films formulated with this mixture. Films containing a mixture of SB + PS significantly inhibited ($p < 0.05$) the growth of PD and PI on DRBC agar. In this work, they found that the use of mixtures of PS + SP, SB + PS, and SB + SP incorporated to HPMC-lipid films resulted in smaller inhibition zones ($p < 0.05$) than the use of films containing PS or SB alone. Thus, they concluded that there was not an additive effect for the inhibition of PD or PI. Among all organic acid salts tested, they found that potassium sorbate (PS) and sodium benzoate (SB) were the most effective salts in controlling both PD and PI.

CHAPTER 3

THEORY

In this study, the diffusion coefficient, partition coefficient and crystal dissolution constant of Psb in the films were determined by using the experimental data obtained in the release kinetics measurements with the mathematical model presented below. In a typical release test, active agent incorporated polymer film with a thickness of L is placed in a limited volume of solution which is well stirred. If it is assumed that there is no chemical reaction between the active compound and the film, mass transfer in the film takes place only by diffusion, the diffusion coefficient of active compound in the film, D, is constant and the overall release of the active compound controlled by Fickian diffusion, then the change in the concentration of active compound in the film with respect to time and position is given by Fick's Second law as follows:

$$\frac{\partial C_f}{\partial t} = D\frac{\partial^2 C_f}{\partial x^2} \tag{3.1}$$

One initial and two boundary conditions are required for the solution of Eq. 3.1. In this study, one side of the film is made impermeable for the desorption of active compound into the solution as it is indicated in the following figure.

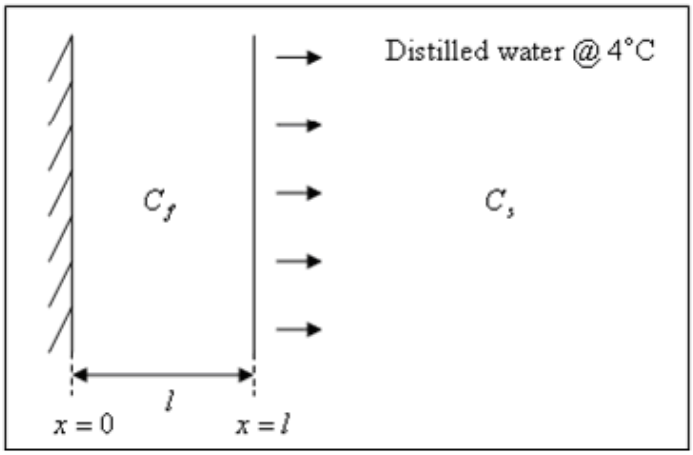


Figure 3.1. Schematics of the mathematical model.

Thus:

$$x = 0 \qquad \frac{\partial C_f}{\partial x} = 0 \tag{3.2}$$

The rate at which active compound leaves the film from the upper surface is always equal to that at which it enters the solution. This condition is represented by

$$x = L \qquad V_{sol} \frac{\partial C_s}{\partial t} = -DA_m \frac{\partial C_f}{\partial x} \tag{3.3}$$

Eq. 3.3 indicates the fact that the concentration of active compound in the solution depends only on time since the solution is well stirred, thus, its total amount in the solution and in the film remains constant as diffusion proceeds. If the concentration within the surface of the film is related to that in the solution through a partition coefficient, *K*,

$$x = L \qquad C_f = KC_s \tag{3.4}$$

then, Eq. 3.3 is rewritten as follows:

$$x = L \qquad a\frac{\partial C_f}{\partial t} = -D\frac{\partial C_f}{\partial x} \tag{3.5}$$

where a is given by

$$a = \frac{V_{sol}}{KA_m} \tag{3.6}$$

Initially, the antimicrobial agent is entrapped into the polymer film at a uniform concentration of C_o. Then, the initial condition is

$$t = 0 \qquad C_f = C_0 \tag{3.7}$$

A solution of these model equations from Eq. 3.1 through Eq. 3.7 is presented in a classical book of Crank (1975). In a form expressing the ratio of total amount of active compound desorbed from the film at any time t, Mt, to the amount desorbed at equilibrium, M_∞, the solution is

$$M_{Fickian} = M_\infty\left[1 - \sum_{n=1}^{\infty}\frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n}\exp(-Dq_n{}^2 t / L^2)\right] \tag{3.8}$$

where $\alpha = \frac{a}{L}$ and the q_n's are the non-zero positive roots of

$$\tan q_n = -aq_n \tag{3.9}$$

Using this analytical solution, the diffusivity of active compound in the film was determined through nonlinear least square analysis by minimizing the difference between Eq. 3.8 and experimental uptake curves.

In cases where one stage sorption controlled by Fickian diffusion was observed, Eq. 3.8 was used to determine the diffusivity of active compound in the film by minimizing the difference between the experimental uptake curves and model predictions.

Two stage sorption is defined as anomalous type of sorption where the sorption curve is composed of two different parts: 1) fast Fickian sorption; 2) slow non – Fickian sorption. The sorption curve is initially Fickian until it starts to level off which results from the release of dissolved potassium sorbate particles. Instead of reaching the equilibrium level which is typical for Fickian sorption, the curve is extended through a non – Fickian part. In this study, the second non – Fickian sorption is observed due to slow dissolution of crystals.

Berens and Hopfenberg (1978) have proposed a theory describing the features of "two – stage" sorption. The theory assumes that overall sorption is described by two phenomenologically independent contributions: a diffusion part $M_{Fickian}$ governed by Fick's law and a structural part resulting from polymer relaxation. The total weight loss/gain at any time t is then expressed as a linear superposition of these contributions. This model has been adopted in this study, thus, in the case of two stage sorption, the total weight loss is defined as:

$$M_{Total}(t) = M_{Fickian}(t) + M_{Dissolution}(t) \tag{3.10}$$

where the weight loss due to dissolution of crystals is described by the following expression.

$$M_{Dissolution} = M_{\infty}(1 - \exp(-kt)) \tag{3.11}$$

CHAPTER 4

MATERIALS AND METHODS

4.1. Materials

Cellulose acetate with a molecular weight of 50,000 and acetyl content of 39.8% was obtained from Eastman (Kingsport, TN, USA). Acetone (99 %) was obtained from Merck (Darmstadt, Germany). Potassium sorbate was obtained from AppliChem. For antimicrobial tests, *Penicillium commune* (NRRL 890, NITE Biological Resource Center (NBRC), Japan) was used as a test organism for the antimicrobial tests. Potato dextrose agar (PDA) from Oxoid was used as growing medium. Peptone water and tween 80 were purchased from Fluka. Tartaric acid and glycerol were obtained from Merck.

4.2. Methods

4.2.1. Preparation of Films

4.2.1.1. Preparation of Single Layer Films

To prepare single layer films, potassium sorbate (Psb) and cellulose acetate (CA) were dissolved in water and acetone, respectively. Then, Psb/water solution was poured into CA/acetone solution under stirring. The stirring of the mixture continued until all the Psb solution is added and dissolved into the CA solution. Once the homogeneous

mixture was obtained, the solution was left standing for 24h to eliminate bubbles. The solution was then cast on a glass support with the aid of an automatic film applicator (Sheen, Automatic film applicator-1133N, Kingston, England) at a speed of 100 mm/sec. The thickness of the film was adjusted by a four-sided applicator with the gap size of 300 or 500 micron. Immediately after casing, the film was placed into an environmental chamber (Siemens, Simatic OP7, Massa Martana, Italy) and dried for 1h at 25°C or 50°C and 40 % relative humidity. Then the films were peeled out from the glass support and further dried in a vacuum oven at 100°C for a period of 24h. Psb concentration in the film forming solution was kept constant at 2% (w/w) while CA (10%, 12.5%, 15% by weight), acetone and water concentrations were changed.

4.2.1.2. Preparation of Multilayer Films

The casting solutions of the first and third barrier layers were prepared by dissolving 15% (w/w) CA in acetone while the casting solution of the middle layer was prepared in the same way as described in section 4.2.1.1. After waited 24h for the precipitation of micro bubbles, the solutions were cast subsequently on a glass support. The first layer was cast on a glass support with the aid of an automatic film applicator. After 2 minutes drying, the second layer was cast on top of the first one and after 8 minutes drying, finally the third layer was cast and the films were dried for 1h at 25°C or 50°C, 40% relative humidity in environmental chamber (Siemens, Simatic OP7, Massa Martana, Italy). After removing from the chamber, the films were placed into a vacuum oven at 100°C and kept there for 24h.

4.2.2. Release Tests

The release tests were conducted for both dense and porous sides of the films separately by using a hand made glass apparatus as indicated in Figure 4.1. In this apparatus, film samples 5 cm in diameter were squeezed between two silicon sealing

ring one of which supported by a glass support and the other one contains a circular hole with a diameter of 5 cm at the center. To prevent contact of water with the protected side of the film, the silicon ring supported by the glass was surrounded with paraffin. The apparatus was then placed into glass Petri dish (10 cm in diameter) containing 100 ml distilled water with pH 7 at 4°C and stirred magnetically at 240 rpm with a 2 cm long Teflon coated rod. The release of Psb to the release medium was monitored by taking 100µl samples at different time periods. After diluting with 1 ml distilled water, the absorbance of the samples was measured at 254 nm in UV spectrometer (Perkin Elmer Model No: Lambda 45).

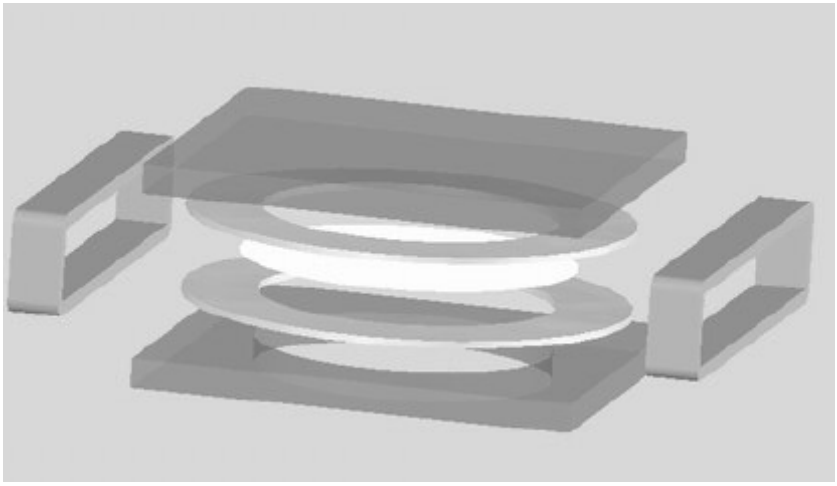


Figure 4.1. The illustration of the experimental set-up used in release tests of porous or dense surfaces of cellulose acetate films.

4.2.3. Morphological Characterization of Films

Morphology of the films was examined by scanning electron microscopy (SEM) on a Philips XL-30SFG model. Energy dispersive X-ray spectroscopy (EDX) was used to make elemental analysis of the samples using same SEM device. For both SEM and EDX analysis, samples were coated with gold palladium using a Magnetron Sputter Coating Instrument.

4.3. Antimicrobial Tests

4.3.1. Preparation of PDA Agars

39 g PDA was dissolved in 1 L distilled water and the solution was stirred and boiled until it was completely dissolved. Upon dissolution, PDA solution was cooled down to 25°C and its pH was adjusted to 5.6 by 0.1M NaOH and 0.1M HCl solutions. After that, it was sterilized in an autoclave at 121°C for a period of 15min. Following the sterilization, the temperature of the solution was decreased to 25°C and its pH was adjusted to 3.5 by using sterile tartaric acid (1 g tartaric acid + 10 ml sterile water) solution. At the end, 20 ml of the prepared PDA solutions were poured into Petri dishes and 10 ml of it was poured into slant tubes and stored in the refrigerator.

4.3.2. Preparation of Test Organism

Penicillium commune colonies were transferred form PDA slant and dissolved in 5ml sterile water. The culture was then incubated at 25°C for 2 hours and 100µl portions of it were dispersed onto PDA agar plates. The agar plates were incubated at 25°C for 5 days to develop *P. commune* spores. At the end of this period, the grown *P. commune* spores were harvested by sterile tween-80 solution (0.05% w/v) and added to the PDA slants. These slants were then further incubated at 25°C for 7 days and once more harvested by using sterile tween-80 solution.

4.3.3. Determining the Number of *P. commune* Spores

The 50 ml solution containing *P. commune* spores was centrifuged and the supernatant was discarded. The precipitated pellet was dissolved in sterile peptone

water (0.1% w/v) and then the number of spores was determined. In order to adjust the spore number, 100 µl of sterile peptone water containing precipitated pellet was taken and diluted with 1 ml distilled water and the number of spores were counted under microscope by using a Thoma Counting Chamber. After necessary dilutions the spore number was adjusted to $24x10^6$ spores/ml.

4.3.4. Zone of Inhibition Test

The antimicrobial activity was determined by the classical zone of inhibition test. For this purpose 100 µl of the *P. commune* culture was transferred and spreaded onto PDA agar plates. Then, 1.3 cm diameter discs of films obtained aseptically with cork borer was put in the middle of each Petri dish. The Petri dishes were incubated at 25°C during 7 days and monitored for growth of *P. commune* and zone formation. The diameter of the zones was measured with a caliper at 3^{rd}, 5^{th} and 7^{th} days.

4.4. Mathematical Analysis

In the case of two stage sorption, the release profiles of the active agent are described by the diffusion coefficient, D, and the crystal dissolution constant, k. These two parameters were determined by minimizing the difference between the experimental data and model predictions given by Eq. 3.8 and Eq. 3.11, respectively. For one stage sorption, the weight loss due to crystal dissolution becomes zero and the diffusion coefficient is evaluated by combining Eq. 3.8 with the experimental data.

CHAPTER 5

RESULTS AND DISCUSSION

In this study, cellulose acetate films including potassium sorbate as an antimicrobial agent were prepared. The influences of the initial casting composition, wet casting thickness, drying temperature and number of layers on the morphology of the films and their release kinetics were investigated. The codes of the films which are be used throughout this chapter and corresponding preparation conditions are listed in Table 5.1.

Table 5.1. Types of the films prepared and their codes.

Film Code	Number of Layers	Wet Casting Thickness (µm)	Temperature (°C)	Composition (wt%)
CA1	Single	300	25	10
	Single	300	25	12.5
	Single	300	25	15
CA2	Single	500	25	10
	Single	500	25	12.5
	Single	500	25	15
CA3	Single	300	50	10
	Single	300	50	12.5
	Single	300	50	15
CA4	Multi	300	25	10
	Multi	300	25	12.5
	Multi	300	25	15
CA5	Multi	500	25	10
	Multi	500	25	12.5
	Multi	500	25	15
CA6	Multi	300	50	10
	Multi	300	50	12.5
	Multi	300	50	15

5.1. Morphology of the Films

5.1.1. The Influence of Initial Casting Composition on the Morphology of the Films

In this work, the CA films were prepared by using a phase inversion technique. In this technique, an initially homogeneous solution formed by dissolving CA in its solvent acetone and Psb in water separates into polymer lean and polymer rich phases. This occurs due to fast evaporation of acetone and the increase in concentration of water which acts as a non-solvent for CA due to its limited solubility and finally brings the solution to a thermodynamically unstable point, thus, leads to phase separation. The polymer rich phase forms the matrix of the film, while polymer lean phase constitutes the porous structure of the film after all acetone and water are evaporated. The morphological features of the films prepared by this technique can be changed by varying the processing conditions. Among these conditions, the composition of the initial casting solution was found to have a significant role on the structure of the films (Gemili, et al. 2009). Based on these previous observations, three different casting compositions were selected as shown in Table 5.2.

Table 5.2. The compositions of polymer, solvent, non-solvent and antimicrobial agent in the film forming solutions.

Weight Percentage (%) of Four Components			
Polymer (CA)	Solvent (Acetone)	Non-solvent (Water)	Antimicrobial Agent (Psb)
10	60	28	2
12.5	60	25.5	2
15	60	23	2

The scanning electron microscope (SEM) pictures shown in Figure 5.1 through Figure 5.3 illustrate the influence of the initial casting composition on the structure of

the films cast by using a knife with 300µm thickness and dried at 25°C (CA1). It is clear that the increase in the polymer composition from 10 wt% to 15 wt% leads to the formation of a much more dense structure resulting from a decrease in the pore size and porosity of the film.

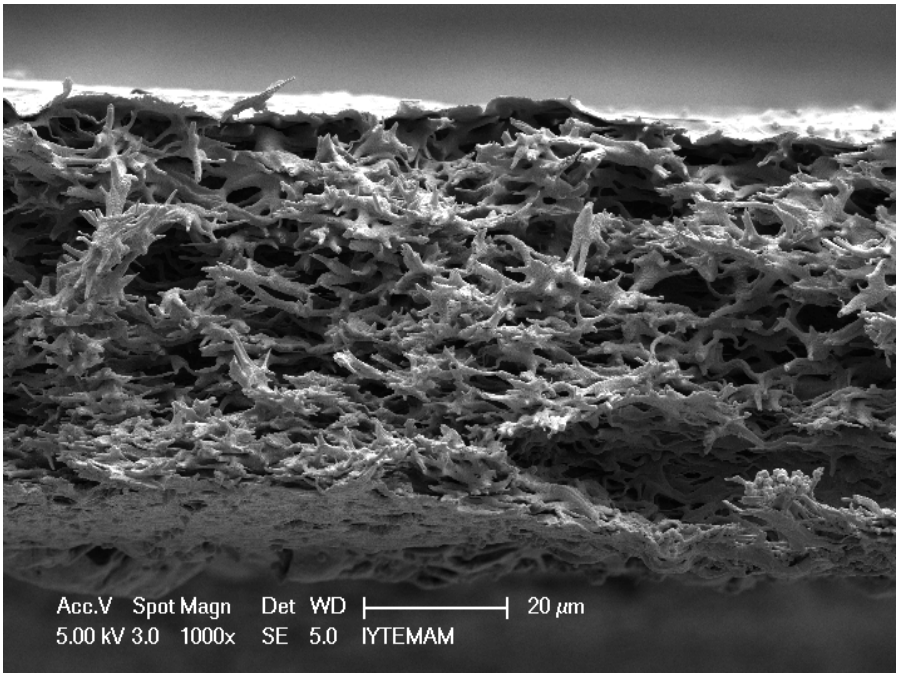


Figure 5.1. SEM of the cross-section of single layer CA film cast with 300µm thickness and dried at 25°C. The CA content in the initial casting solution is 10 wt%. Magnification, 1000x.

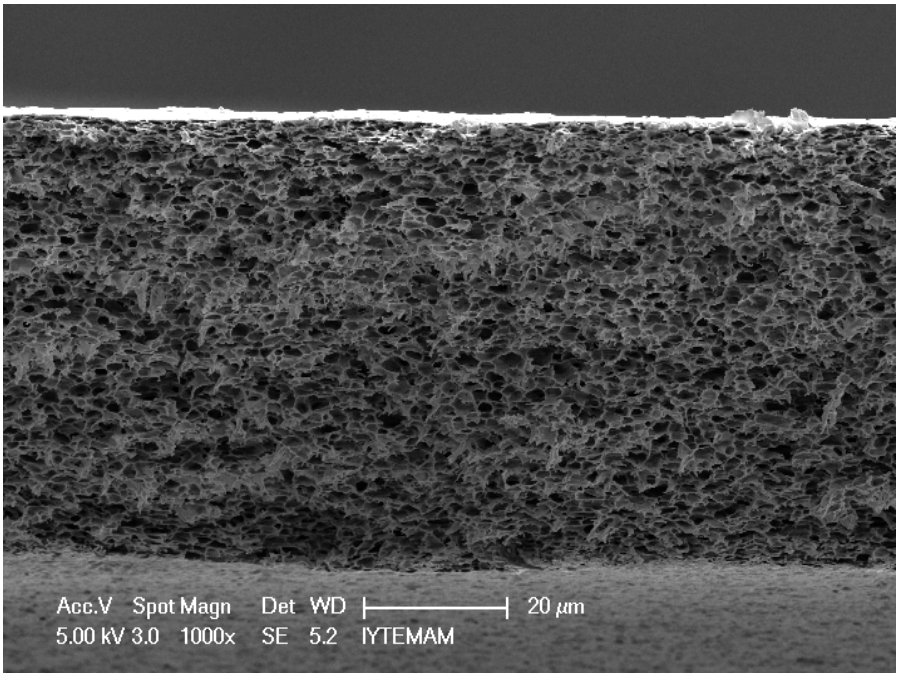


Figure 5.2. SEM of the cross-section of single layer CA film cast with 300μm thickness and dried at 25°C. The CA content in the initial casting solution is 12.5 wt%. Magnification, 1000x.

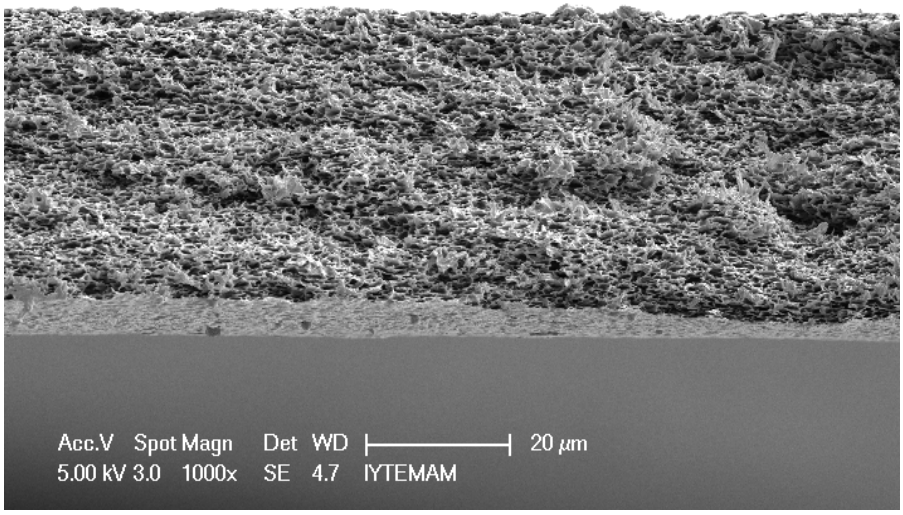


Figure 5.3. SEM of the cross-section of single layer CA film cast with 300μm thickness and dried at 25°C. The CA content in the initial casting solution is 15 wt%. Magnification, 1000x.

In the case of single layer films, drying induced crystallization of potassium sorbate, which is a potassium salt of sorbic acid, was observed as shown in Figure 5.4. After phase separation occurred in the environmental chamber, further drying in the vacuum oven leads to penetration of potassium sorbate into the pores and an increase in its concentration in the pores. This increases supersaturation and induces crystal growth in the pores. Similar phenomena were observed by Caussy (2006) in stones and porous sedimentary rocks which are exposed to sea-salts.

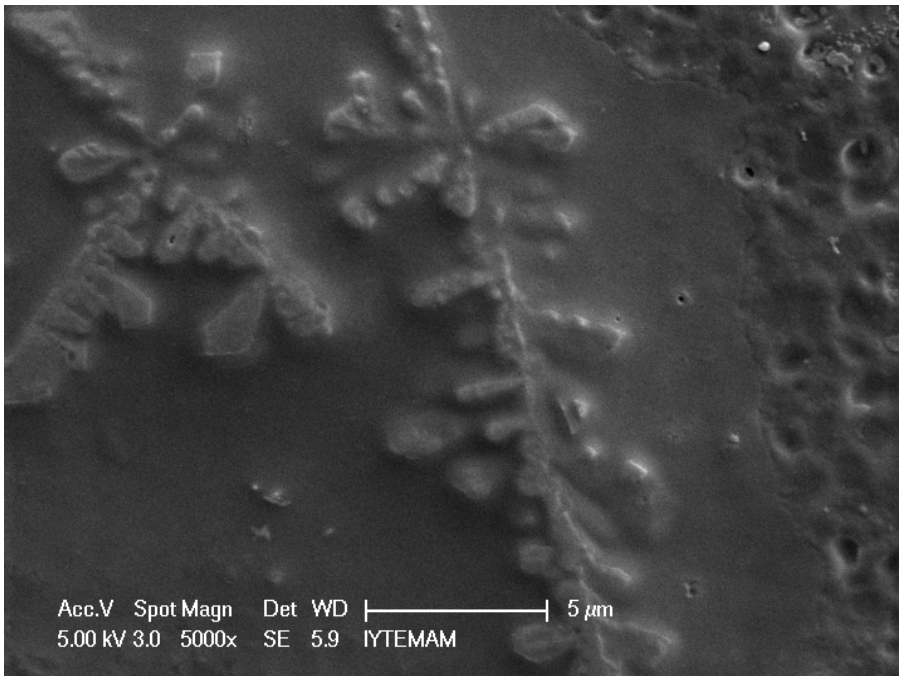


Figure 5.4. An example of a local Psb crystal on the CA film surface. Magnification, 5000x.

Our observations indicate that the initial casting composition of the film forming solution has an important effect on the crystal formation. SEM pictures shown in Figure 5.5 through Figure 5.7 indicate that the size and amount of the crystals formed on the porous surfaces of the films denoted as CA3 decrease as the CA content in the films increases. This result simply indicates the dependence of the crystal formation on the pore size of the films. This observation is in accordance with the conclusion reached by Scherer (2004). He simply considered confined crystallization at the pore scale and found that crystal growth is expected to occur in large pores.

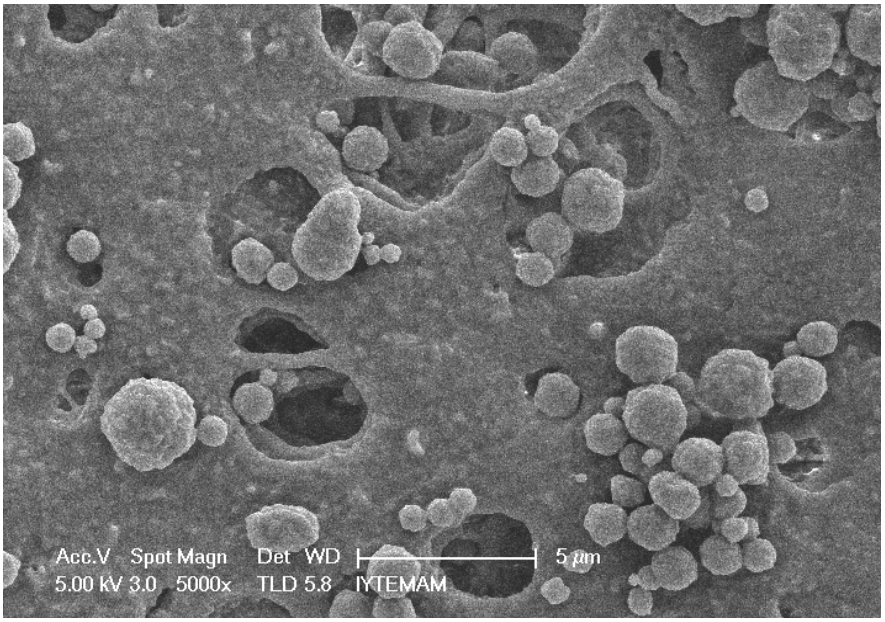


Figure 5.5. SEM picture of crystal taken from the porous side of the CA film cast with 300µm thickness and dried at 50°C. The CA content in the initial casting solution is 10 wt%. Magnification, 5000x.

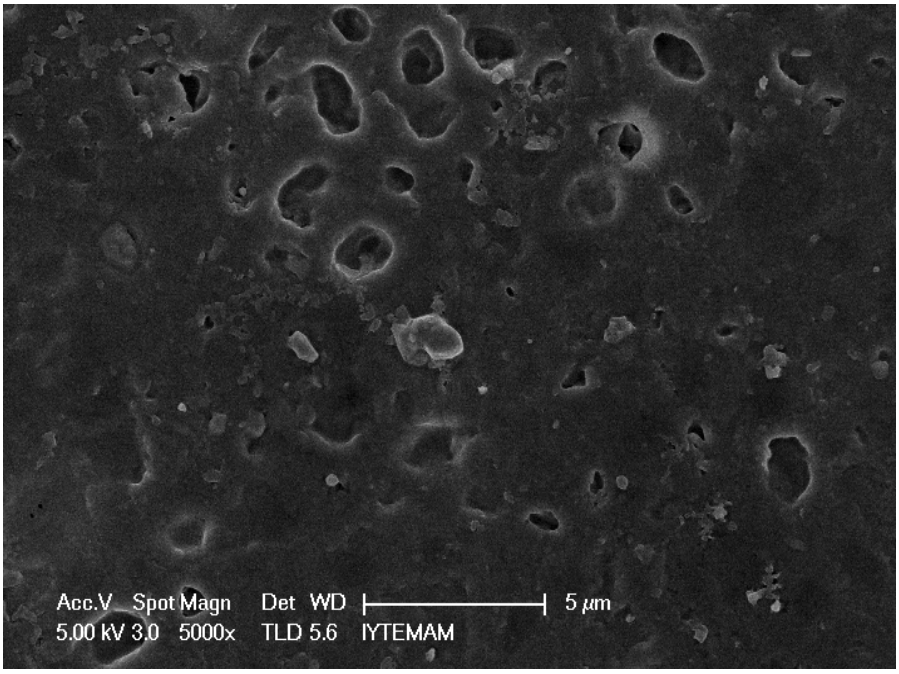


Figure 5.6. SEM picture of crystal taken from the porous side of the CA film cast with 300µm thickness and dried at 50°C. The CA content in the initial casting solution is 12.5 wt%. Magnification, 5000x.

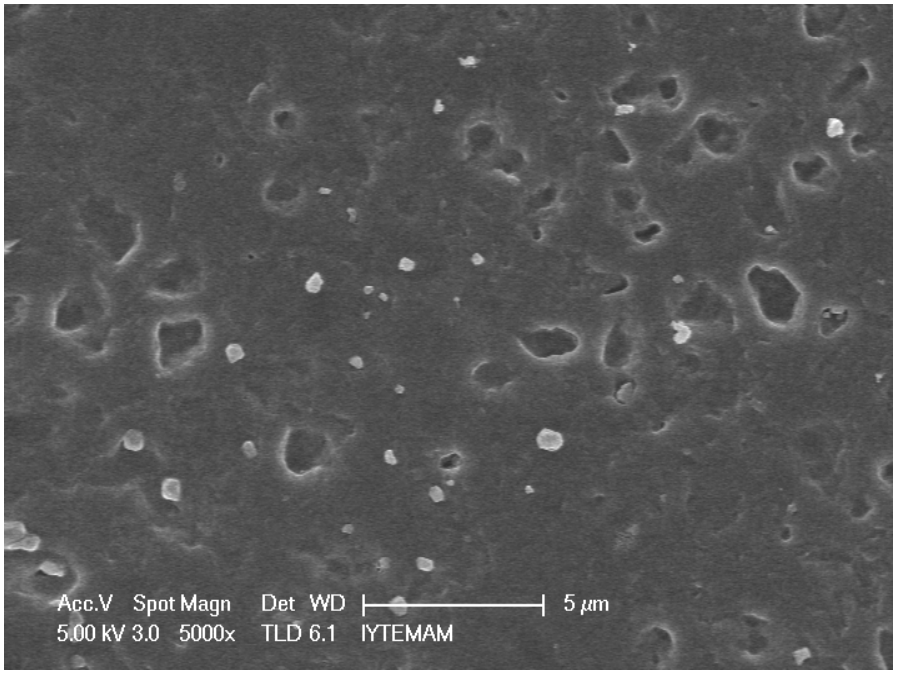


Figure 5.7. SEM picture of crystal taken from the porous side of the CA film cast with 300μm thickness and dried at 50°C. The CA content in the initial casting solution is 15 wt%. Magnification, 5000x.

The films prepared in this study are not only porous but they also have asymmetric structures with a dense skin layer at the top and a porous layer in the bulk as shown in Figure 5.1 through Figure 5.3. This asymmetric structure leads to more crystal formation with larger sizes on the porous surfaces of the films compared to the dense surfaces as illustrated in Figure 5.8 and Figure 5.9.

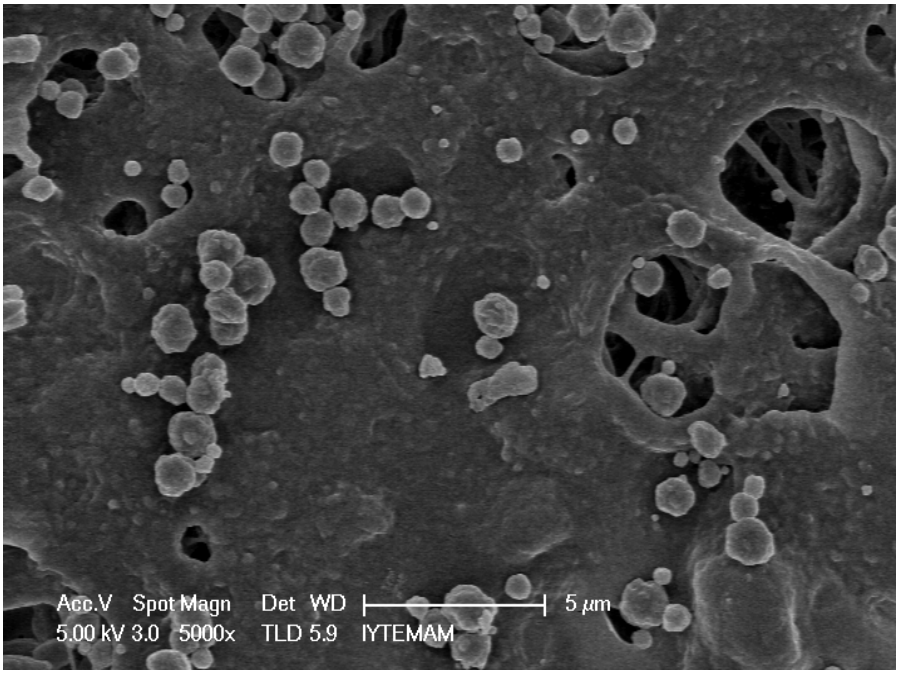


Figure 5.8. SEM picture of crystal taken from the porous side of the CA film cast with 300μm thickness and dried at 25°C. The CA content in the initial casting solution is 10 wt%. Magnification, 5000x.

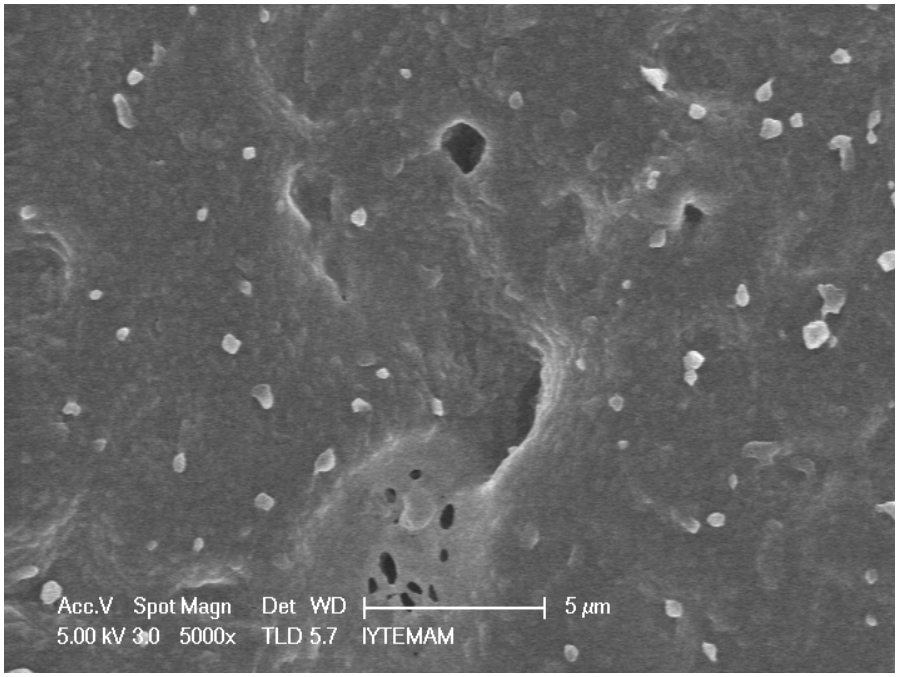
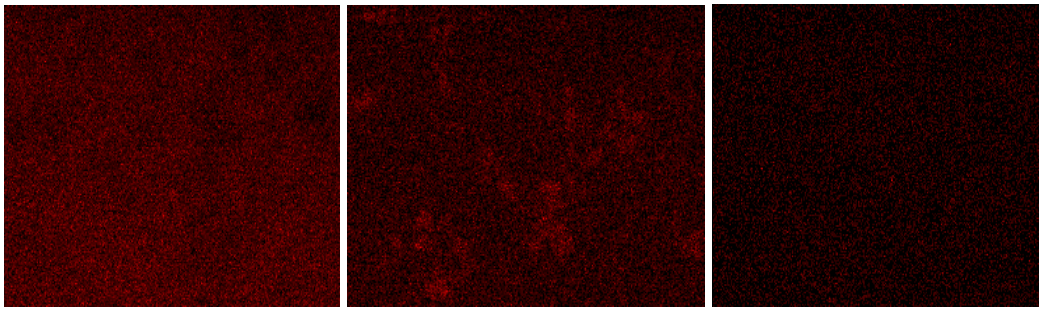


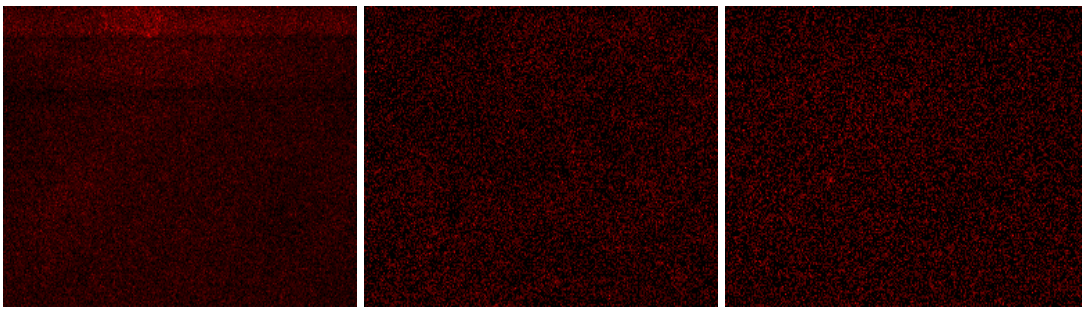
Figure 5.9. SEM picture of crystal taken from the dense side of the CA film cast with 300μm thickness and dried at 25°C. The CA content in the initial casting solution is 10 wt%. Magnification, 5000x.

The presence of Psb crystals in the films was also proved by EDX mapping of same samples. The results shown in Figure 5.10 and Figure 5.11 indicate that the crystals formed on either porous or dense surfaces of the films decrease with the increased polymer concentration. This was observed by the decrease in the red shiny points which represents the crystals. The mapping results also indicate uniform distribution of dissolved Psb particles through dense and porous surfaces of the films.



a) **b)** **c)**

Figure 5.10. EDX mapping of porous side of CA films cast with 300µm thickness and dried at 25°C. The CA contents in the initial casting solution are a) 10wt% b) 12.5wt% c) 15wt%.



a) **b)** **c)**

Figure 5.11. EDX mapping of dense side of CA films cast with 300µm thickness and dried at 25°C. The CA contents in the initial casting solution are a) 10wt% b) 12.5wt% c) 15wt%.

SEM pictures shown in Figure 5.12 through Figure 5.14 illustrate the influence of the initial casting composition on the structure of the multilayer films. Three distinct layers are observed in Figure 5.12 in the case of most porous films prepared with the lowest CA concentration. On the other hand, clear boundaries between the layers disappeared with increased CA content in the films. This may be explained by the

decrease in the pore size and porosity of the middle layer, hence, the structure of the middle layer becomes more similar to the inner and outer layers. Drying induced crystal formation was not observed in multilayer films. This is due to barrier effects of inner and outer layers in transmitting the heat from drying air to the middle layer including Psb. Thus, slower evaporation of the residual volatile compounds from the multilayer film results in a gradual increase in the Psb concentration in the pores which prevents to reach its supersaturation concentration.

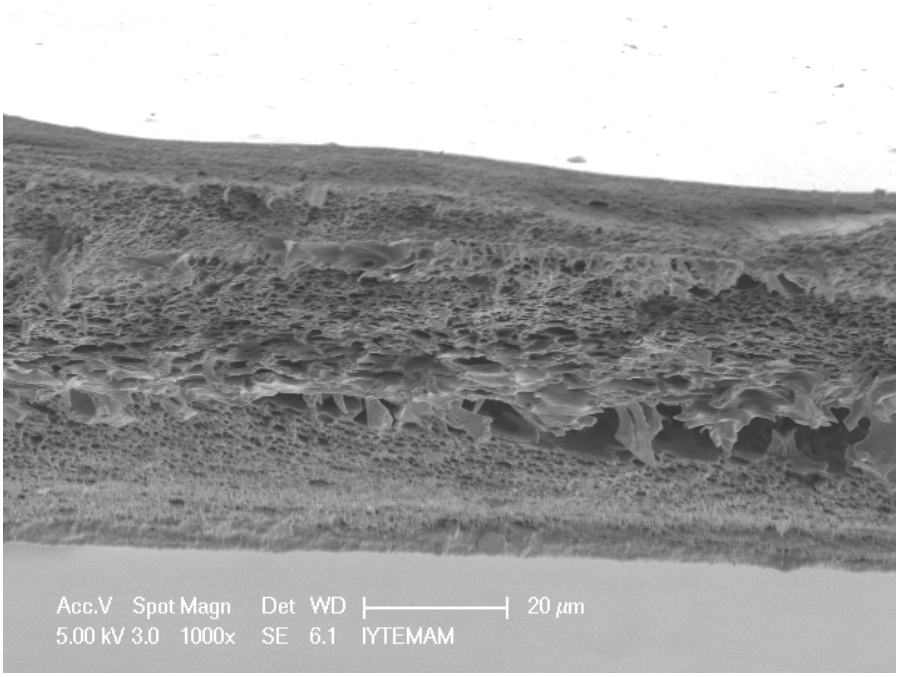


Figure 5.12. SEM of the cross-section of multilayer CA film cast with 300μm thickness and dried at 25°C. The CA content in the initial casting solution is 10 wt%. Magnification, 1000x.

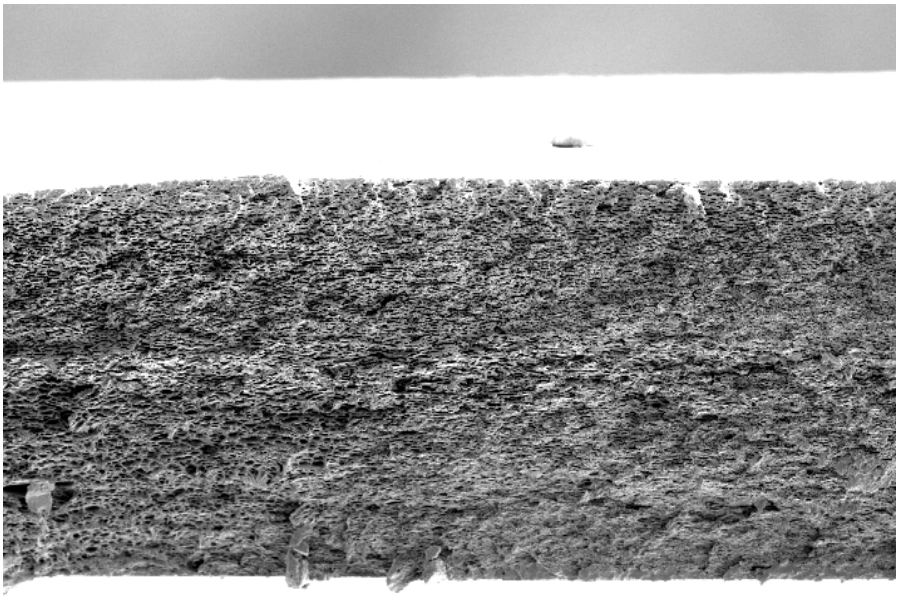


Figure 5.13. SEM of the cross-section of multilayer CA film cast with 300µm thickness and dried at 25°C. The CA content in the initial casting solution is 12.5 wt%. Magnification, 1000x.

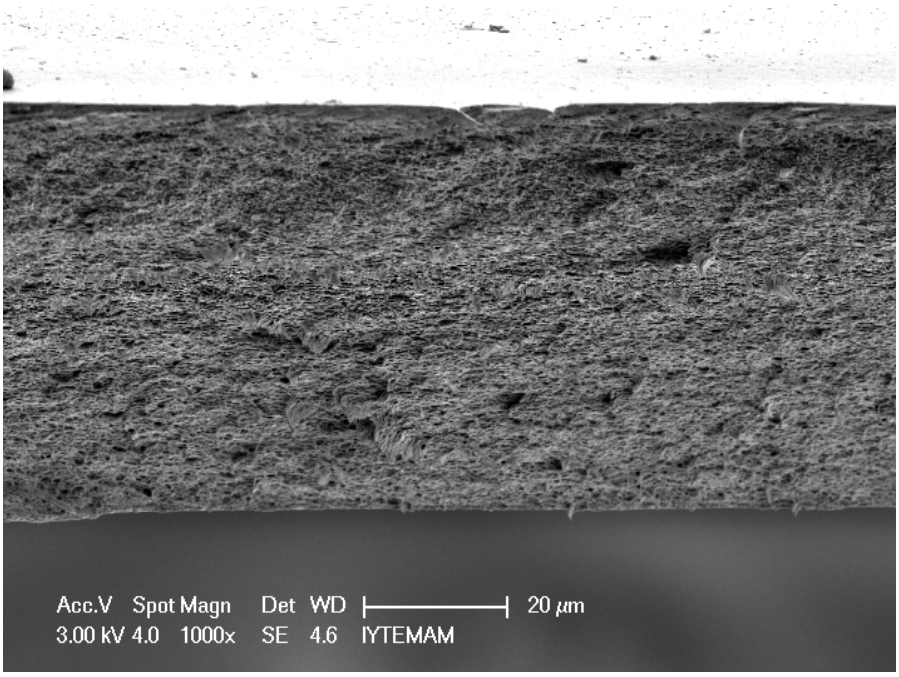


Figure 5.14. SEM of the cross-section of multilayer CA film cast with 300µm thickness and dried at 25°C. The CA content in the initial casting solution is 15 wt%. Magnification, 1000x.

5.1.2. The Influence of Wet Casting Thickness on the Morphology of the Films

Wet casting thickness is considered as another important parameter that influences the morphology of the films, therefore, the films were cast with 300µm and 500µm thicknesses. The increase in the wet casting thickness from 300µm to 500µm for the same single layer composition (15 wt% CA) and drying temperature (25°C) resulted in the formation of a more dense structure with smaller pore sizes and lower porosities. This can be clearly seen by comparing SEM pictures shown in Figure 5.3 and Figure 5.15 respectively.

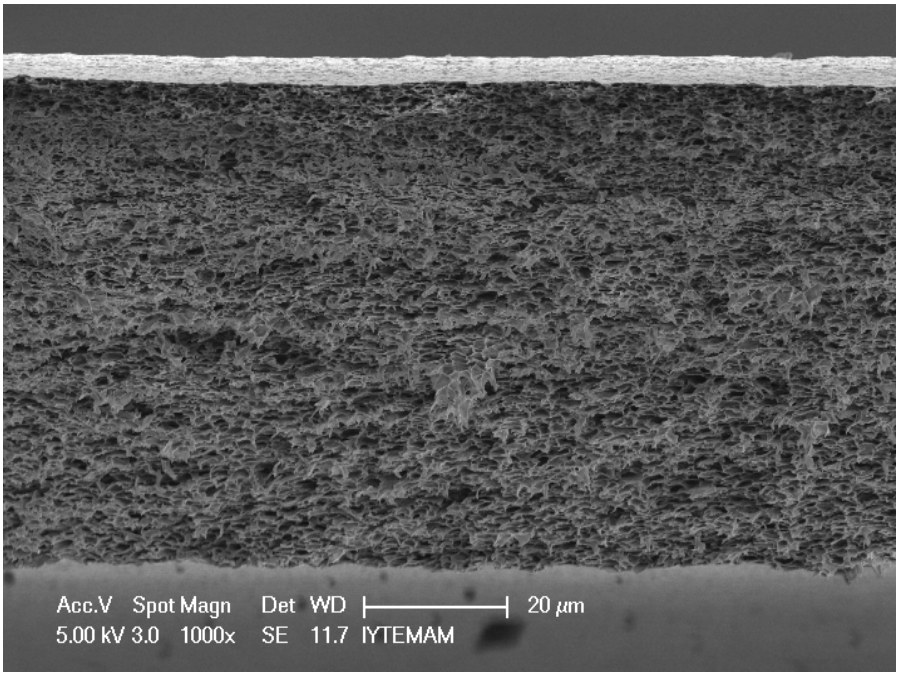


Figure 5.15. SEM of the cross-section of single layer CA film cast with 500µm thickness and dried at 25°C. The CA content in the initial casting solution is 15 wt%. Magnification, 1000x.

Another change in the structure of the films cast with a thicker knife is the decrease in the number of the crystals and the size of the crystals as well as illustrated in Figure 5.16 and Figure 5.17. This observation proves the dependence of crystal formation on the pore size and porosity of the films.

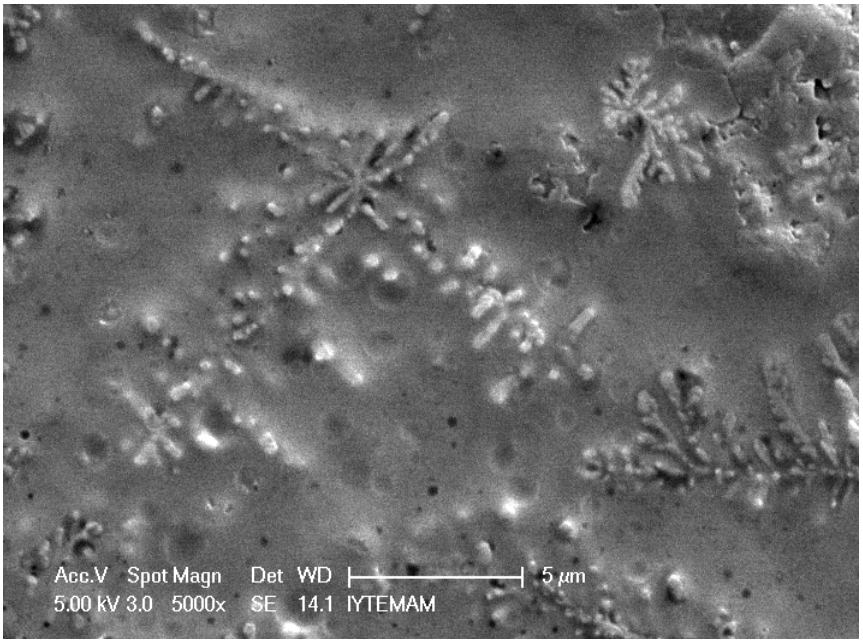


Figure 5.16. SEM picture of crystal taken from the dense side of the CA film cast with 300μm thickness and dried at 25°C. The CA content in the initial casting solution is 15 wt%. Magnification, 5000x.

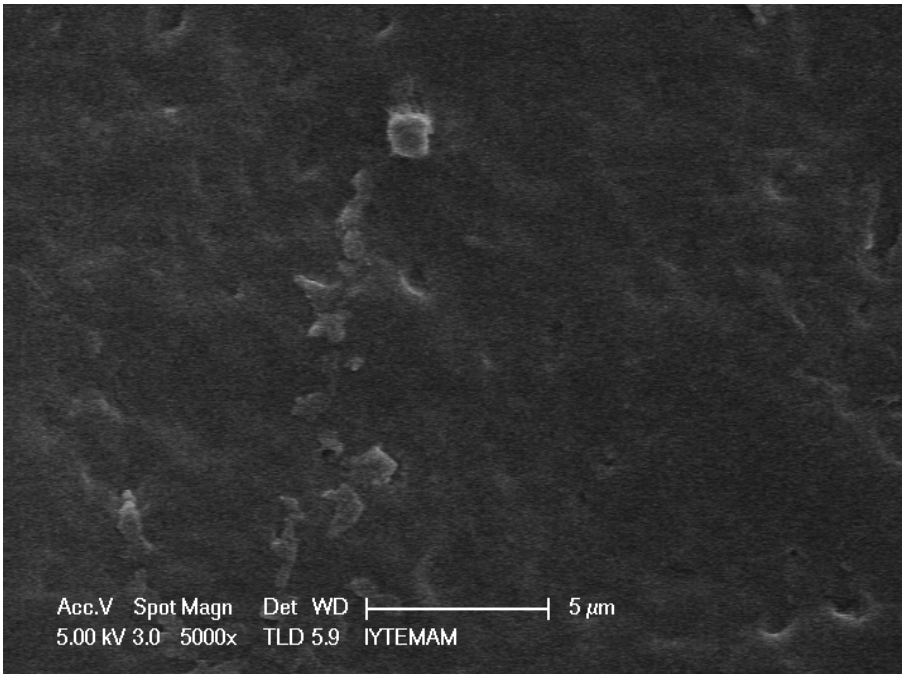


Figure 5.17. SEM picture of crystal taken from the dense side of the CA film cast with 500μm thickness and dried at 25°C. The CA content in the initial casting solution is 15 wt%. Magnification, 5000x.

The comparison of SEM pictures shown in Figures 5.13 and 5.18 clearly indicates that casting the multilayer films with a thicker knife also caused formation of a more dense film structure, consequently, no crystals were detected in the structure.

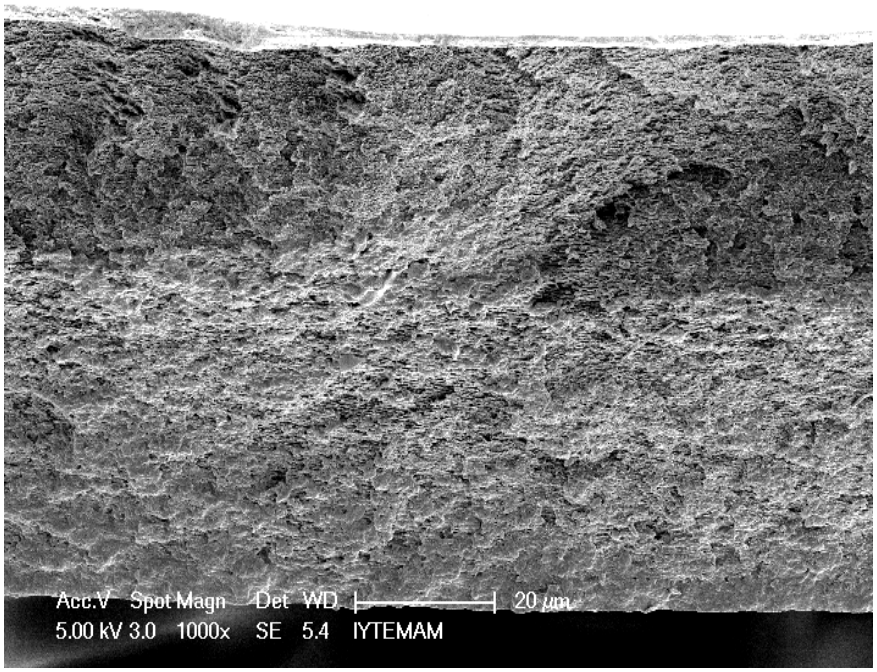


Figure 5.18. SEM of the cross-section of multilayer CA film cast with 500µm thickness and dried at 25°C. The CA content in the initial casting solution is 12.5 wt%. Magnification, 1000x.

5.1.3. The Influence of Drying Temperature on the Morphology of the Films

To investigate the influence of drying temperature on the structure of the films, the temperature in the environmental chamber was raised from 25°C to 50°C. The comparison of SEM pictures shown in Figure 5.2 and Figure 5.19 indicates that the pore size and porosity of the films decreased with the increase in the drying temperature. This observation is in agreement with others. For example, Young et al. (1999) have found a change in the structure of crystalline poly(ethylene-co-vinyl alcohol) from a particulate to a dense morphology with increased evaporation temperature while

Mohamed and Al-Dossary (2003) have measured lower water permeabilities through the membrane prepared under higher evaporation temperature.

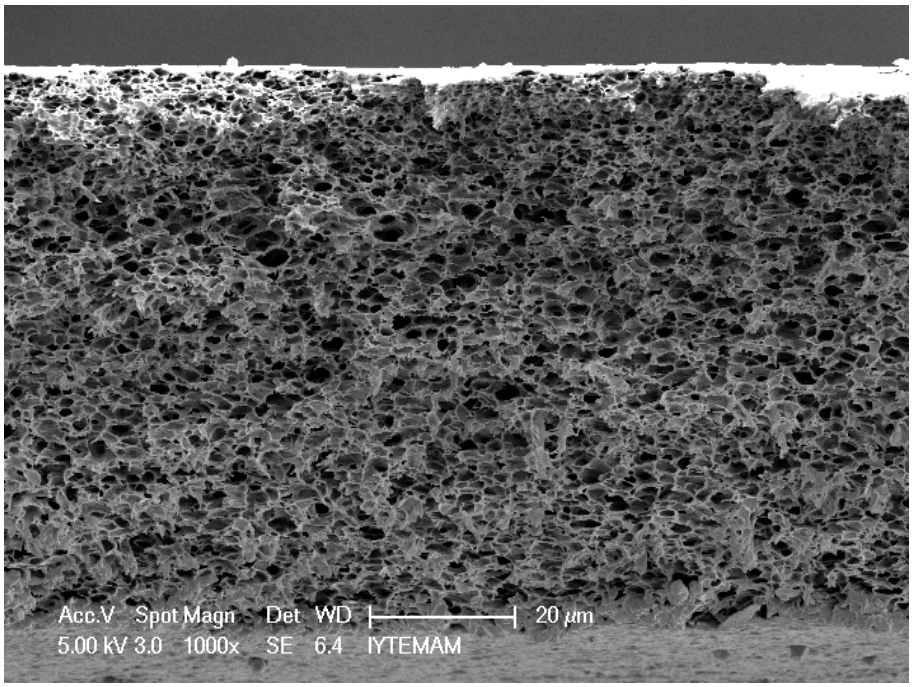


Figure 5.19. SEM of the cross-section of single layer CA film cast with 300µm thickness and dried at 50°C. The CA content in the initial casting solution is 12.5 wt%. Magnification, 1000x.

Although higher drying temperature favors increased crystallization rates, the decrease in the porosity and pore size of the films becomes a more dominant factor in controlling the crystal formation. Thus, the number of crystals formed in the membrane decreased with increased drying temperature as shown by SEM pictures in Figure 5.20 and Figure 5.21.

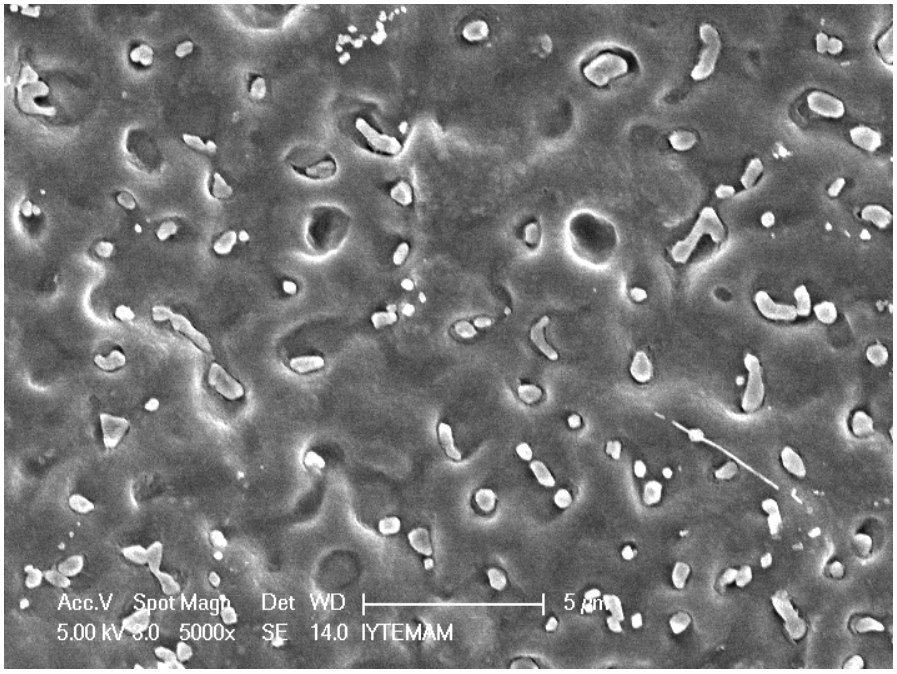


Figure 5.20. SEM picture of crystal taken from the dense side of the CA film cast with 300µm thickness and dried at 25°C. The CA content in the initial casting solution is 12.5 wt%. Magnification, 5000x.

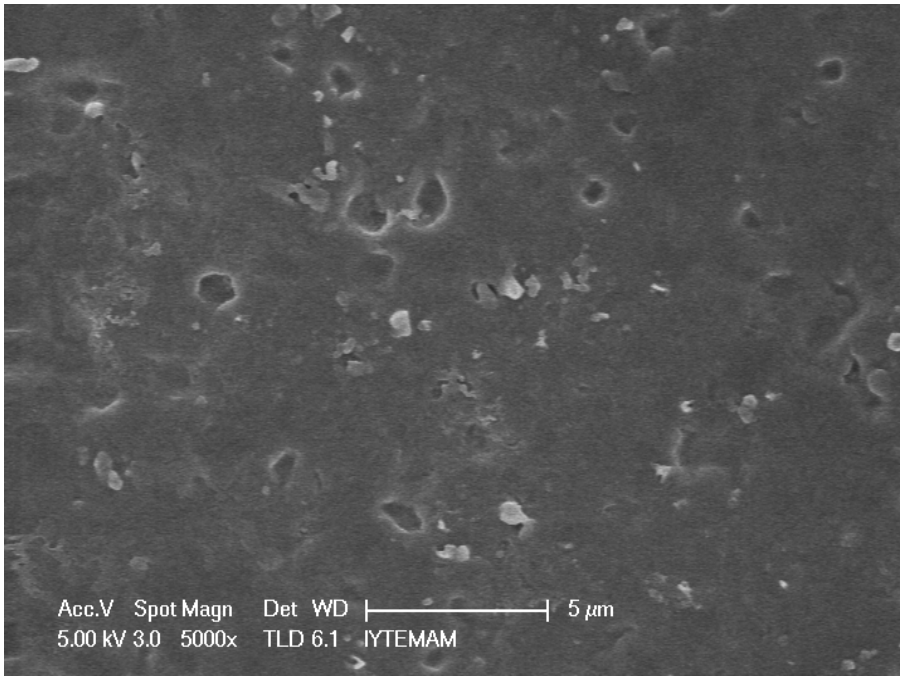


Figure 5.21. SEM picture of crystal taken from the dense side of the CA film cast with 300µm thickness and dried at 50°C. The CA content in the initial casting solution is 12.5 wt%. Magnification, 5000x.

In the case of multilayer films, increasing drying temperature also resulted in the formation of a more dense structure with lower porosity and pore size as seen with the comparison of SEM pictures in Figure 5.13 and Figure 5.22. No crystal formation was observed in the multilayer films prepared under higher drying temperature.

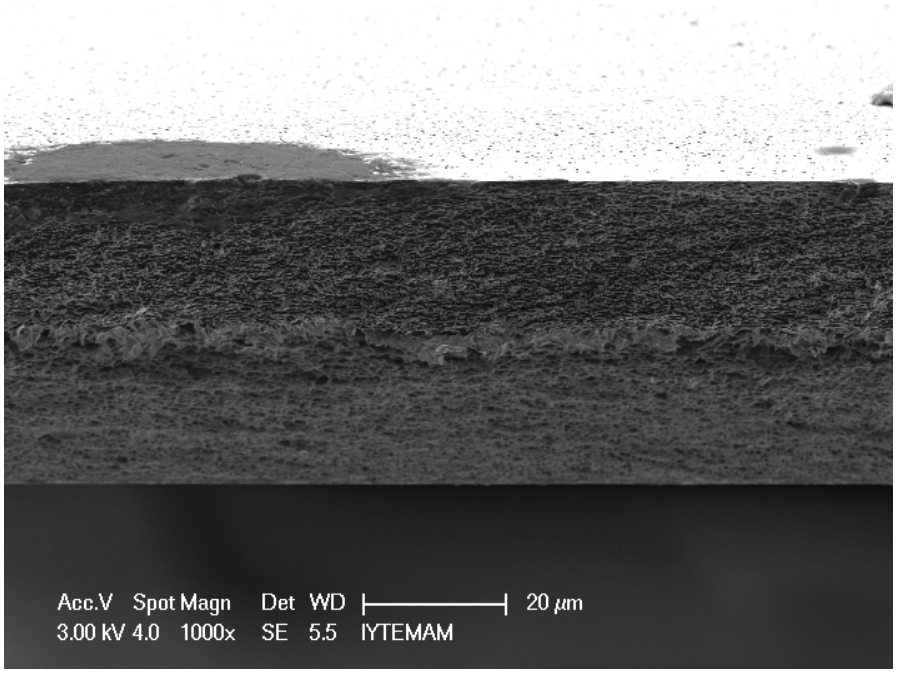


Figure 5.22. SEM of the cross-section of multilayer CA film cast with 300μm thickness and dried at 50°C. The CA content in the initial casting solution is 12.5 wt%. Magnification, 1000x.

5.2. Release Rates of Psb through the Films

In this section, the influence of the initial casting composition, casting thickness and the drying temperature on the release profiles of Psb were discussed. Experimental data plotted in the form of total amount of Psb desorbed from the film at any time *t* as a function of time were evaluated with the mathematical model represented by equations 3.1 through 3.11. The diffusivity of potassium sorbate and the crystal dissolution constant were determined by minimizing the difference between equation 3.8 and 3.10 and experimental data, using solver tool in Excel. The partition coefficients were calculated from the difference in the equilibrium and the initial concentration of Psb in

the solution. The list of the diffusivity, partition coefficient and the crystal dissolution constant is given in Table 5.3 and the influences of each preparation condition on these parameters are discussed in the following sections.

5.2.1. The Influence of Initial Casting Composition on the Release Rates

Figure 5.23 through Figure 5.25 show the release profiles of the single layer films cast with 300μm thickness and dried at 50°C.

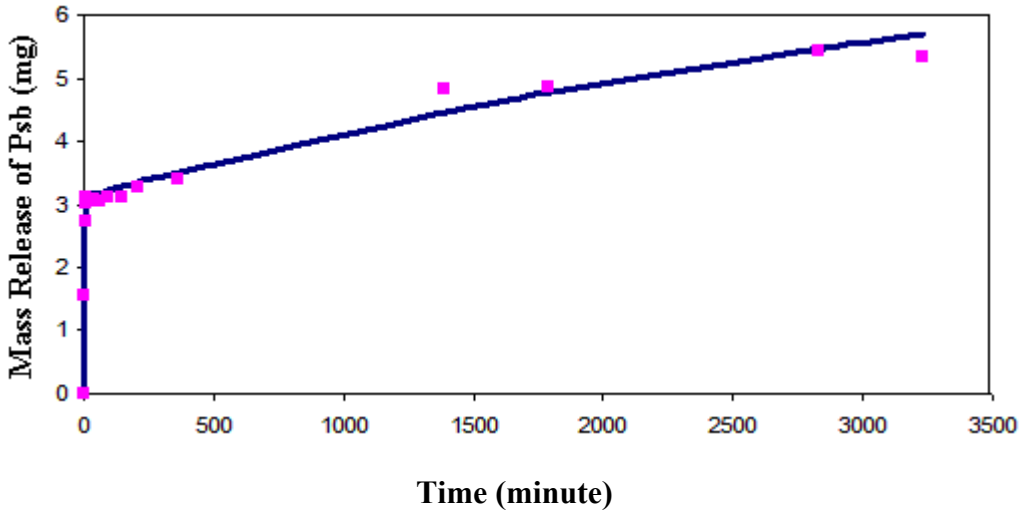


Figure 5.23. The release profile of Psb from the porous side of the single layer CA film cast with 300μm thickness and dried at 50°C. The CA content in the initial casting solution is 10 wt%.

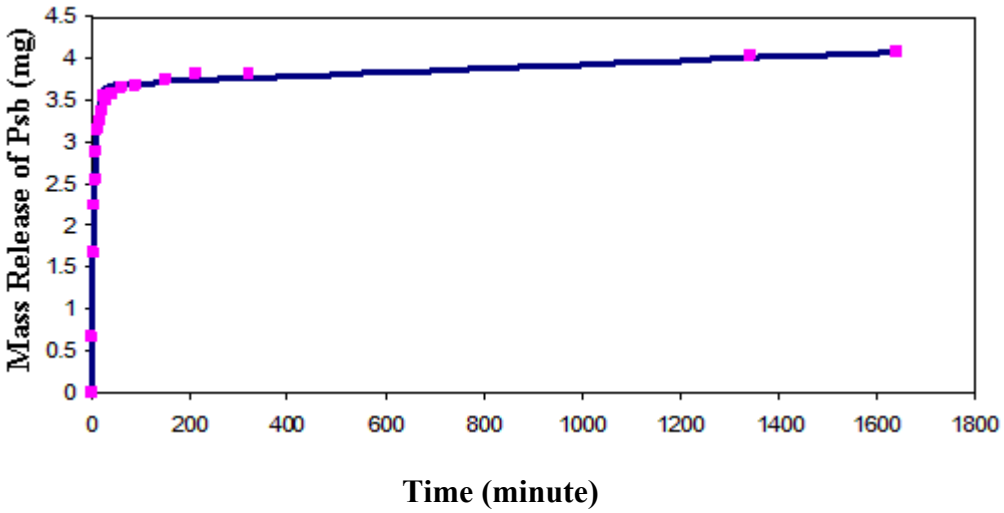


Figure 5.24. The release profile of Psb from the porous side of the single layer CA film cast with 300µm thickness and dried at 50°C. The CA content in the initial casting solution is 12.5 wt%.

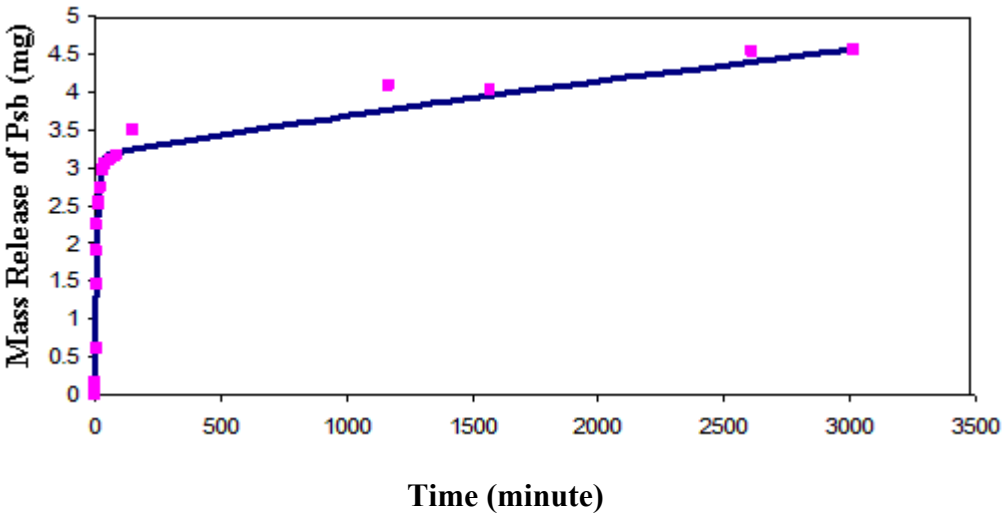


Figure 5.25. The release profile of Psb from the porous side of the single layer CA film cast with 300µm thickness and dried at 50°C. The CA content in the initial casting solution is 15 wt%.

For each film composition, the initial release characteristics follow a Fickian behavior due to release of uniformly distributed dissolved Psb particles. On the other hand, the release rate at longer times is controlled by dissolution of Psb crystals. As a result, a two stage sorption behavior is observed. The results in Table 5.3 indicate that in the case of single layer films, the diffusion coefficient of Psb decreased with increased CA content in the films for each case investigated. This is due to a change in the

structure of the films from porous to dense one, hence, increased mass transfer resistance to the diffusion of Psb. SEM pictures shown in the previous section illustrate that the single layer films are not only porous but they also have different pore sizes at the top (dense surface) and bottom (porous surface) surfaces of the films. Due to their asymmetric structure, release studies were conducted separately for both dense and porous sides of the films. As expected, the diffusion coefficient of Psb was found to be lower when the dense surface was in contact with water.

Table 5.3. Diffusion, partition coefficient and crystal dissolution constant of Psb obtained with the CA films prepared under different conditions.

Film Code	Composition (wt%)	Diffusion Coefficient (cm^2/sec)		Partition Coefficient (cm^3 solution/cm^3 film)		Dissolution Constant (1/minute)	
		Porous Side	Dense Side	Porous Side	Dense Side	Porous Side	Dense Side
CA1 300µm* 25°C**	**10**	9.65×10^{-8}	4.23×10^{-8}	426	474	2.03×10^{-4}	4.47×10^{-4}
	12.5	3.07×10^{-8}	2.33×10^{-8}	577	613	2.03×10^{-4}	2.34×10^{-4}
	15	1.72×10^{-8}	1.52×10^{-8}	664	824	1.29×10^{-4}	1.65×10^{-4}
CA2 500µm 25°C	**10**	3.3×10^{-8}	3.08×10^{-8}	399	360	1.02×10^{-4}	4.37×10^{-4}
	12.5	2.35×10^{-8}	1.33×10^{-8}	423	435	1.03×10^{-4}	1.88×10^{-4}
	15	1.52×10^{-8}	8.68×10^{-9}	585	590	1.32×10^{-4}	0.9×10^{-4}
CA3 300µm 50°C	**10**	7.35×10^{-8}	3.25×10^{-8}	389	470	2.11×10^{-4}	2.08×10^{-4}
	12.5	2.52×10^{-8}	1.62×10^{-8}	493	612	2.05×10^{-4}	3.85×10^{-4}
	15	1.62×10^{-8}	1.35×10^{-8}	833	974	1.21×10^{-4}	2.38×10^{-4}
CA4 300µm 25°C	**10**	1.42×10^{-9}	9.9×10^{-10}	296	320	-----	------
	12.5	9.02×10^{-10}	6.18×10^{-10}	323	403	-----	-----
	15	5.65×10^{-10}	8.18×10^{-10}	399	530	-----	-----
CA5 500µm 25°C	**10**	9.18×10^{-10}	4.18×10^{-10}	422	510	-----	-----
	12.5	4.85×10^{-10}	2.68×10^{-10}	511	486	-----	-----
	15	3.02×10^{-10}	1.85×10^{-10}	525	523	-----	-----
CA6 300µm 50°C	**10**	1.41×10^{-9}	3.25×10^{-10}	315	416	-----	-----
	12.5	5.68×10^{-10}	2.52×10^{-10}	512	490	-----	-----
	15	7.68×10^{-10}	4.68×10^{-10}	506	581	-----	-----

*: Initial casting thickness of the film forming solution. **: Drying temperature of the film forming solution.

The release rate at later stages is determined by the crystal dissolution constant. It was found that the crystal dissolution constant decreased slightly or did not change at all with the increased CA content in the films. The highest dissolution rate constants were obtained with the most porous film structures which allow fast penetration of water into the film, thus, dissolution of crystals at a fast rate. When the crystal dissolution constant does not change with the CA content in the films, the decrease in the rate of diffusion of water is compensated by the presence of smaller crystals in the film which can be dissolved at a faster rate. It is noted that in most cases the crystals present on the dense surfaces of the films dissolve at a higher rate than those which exist on the porous surfaces. This is due to the presence of smaller crystals on the dense surfaces compared to those on the porous surfaces of the films as shown previously by SEM pictures in Figure 5.8 and Figure 5.9, respectively.

The initial casting composition was found to have an influence on the partition coefficients of Psb in single layer films. A change in the structure from porous to dense with increased CA content in the films caused immobilization of more Psb in the films. For the same film composition, the partition coefficient of Psb was found to be higher on the dense sides of the films due to presence of smaller pores.

Figure 5.26 through Figure 5.28 show representative release profiles for multilayer films cast with 500μm thickness and dried at 25°C. One stage release of Psb which follows Fickian behavior was observed for all multilayer films prepared since the crystal formation was not observed in these films. The results listed in Table 5.3 indicate that the diffusion coefficients of Psb through multilayer films decreased by almost two orders of magnitude due to increased mass transfer resistances with the addition of inner and outer layers. The decrease in the diffusivities with increased CA content is smaller than that observed for single layer films. Although the release rate of Psb from the porous surfaces was found to be higher as reflected by higher diffusivities, the difference in the release rates measured from each surface is small. For each multilayer film prepared, the partition coefficient of Psb increased with the increased CA content in the films. In addition, the affinity of Psb to the dense surface was found to be slightly higher. It is interesting to note that in most cases, the amount of Psb immobilized in the single layer films is higher compared with the amount immobilized in multilayer films. At the first glance, this seems to be a contradictory result since multilayer films have more dense structures and Psb has a higher affinity to dense structures. However, in the case of single layer films, Psb is entrapped not only in the

porous matrix but also in the undissolved crystals as well, causing more Psb to be entrapped in the film when equilibrium is reached.

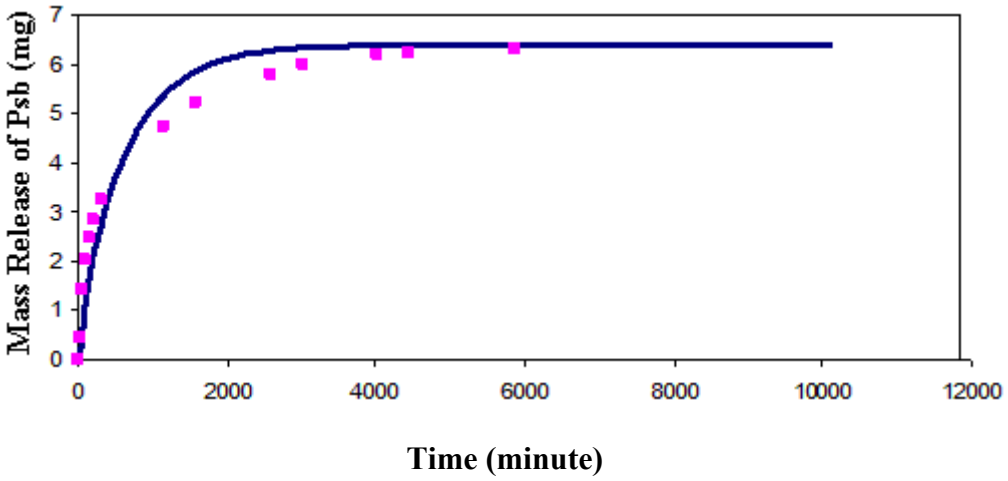


Figure 5.26. The release profile of Psb from the porous side of the multilayer CA film cast with 500µm thickness and dried at 25°C. The CA content in the initial casting solution is 10 wt%.

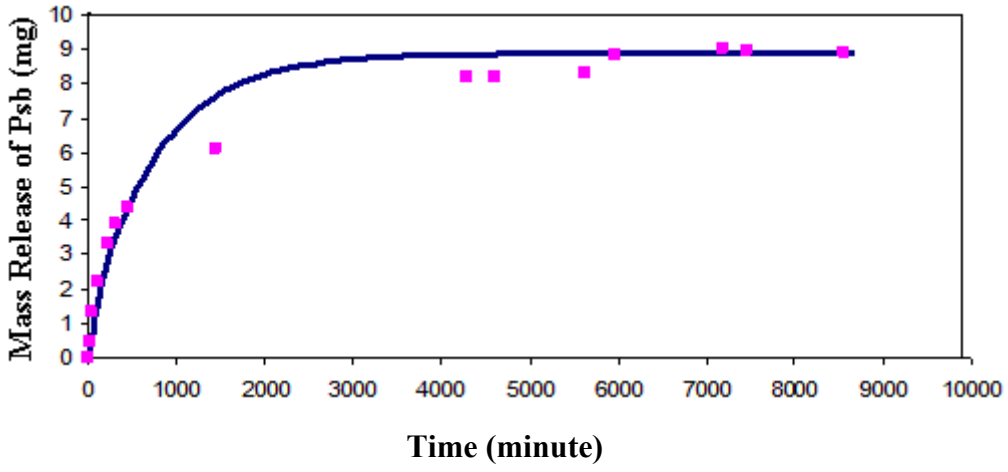


Figure 5.27. The release profile of Psb from the porous side of the multilayer CA film cast with 500µm thickness and dried at 25°C. The CA content in the initial casting solution is 12.5 wt%.

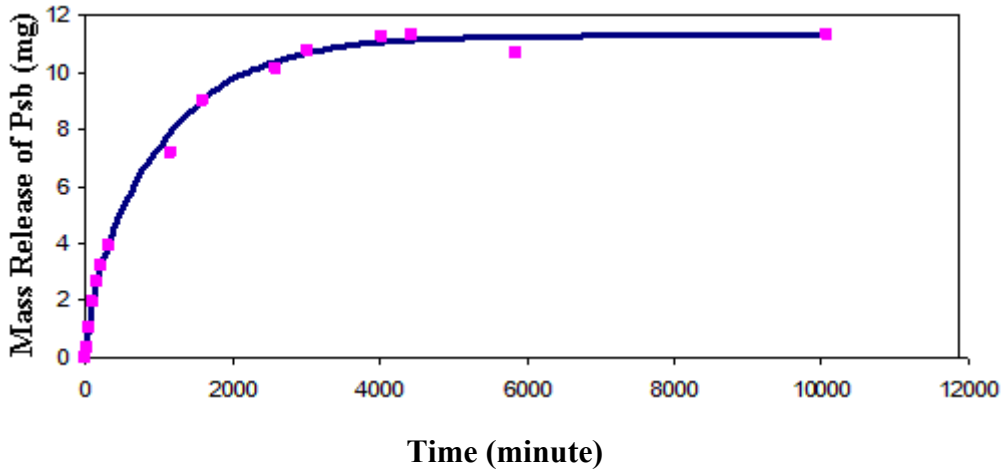


Figure 5.28. The release profile of Psb from the porous side of the multilayer CA film cast with 500μm thickness and dried at 25°C. The CA content in the initial casting solution is 15 wt%.

5.2.2. The Influence of Wet Casting Thickness on the Release Rates

Figure 5.29 through Figure 5.32 show typical release profiles of Psb from single and multilayer films cast with 300 and 500μm thicknesses and dried at 25°C. Increasing the casting thickness did not cause any change in release characteristics, i.e., the release rate from the single layer films is controlled by combined effects of Fickian diffusion and crystal dissolution while one stage sorption controlled by Fickian diffusion was observed in the case of multilayer films. A decrease in the overall porosity and pore size of the films cast with a thicker knife caused slower diffusion of Psb through the film, consequently the time to reach equilibrium increased. In addition, the rate of penetration of water into the polymer matrix became slower leading to slower dissolution of crystals as indicated by a decrease in the crystal dissolution constants. Although the films cast with a larger thickness have more dense structures, the presence of less number of crystals with smaller sizes limits the amount of Psb entrapped in the films, thus, the partition coefficients of Psb decreased with the increased wet casting thickness.

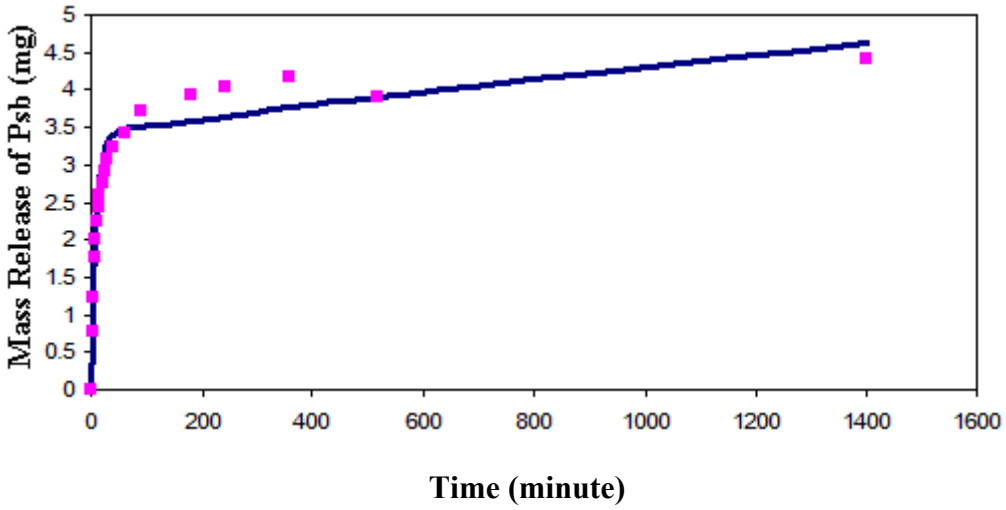


Figure 5.29. The release profile of Psb from the dense side of the single layer CA film cast with 300µm thickness and dried at 25°C. The CA content in the initial casting solution is 12.5 wt%.

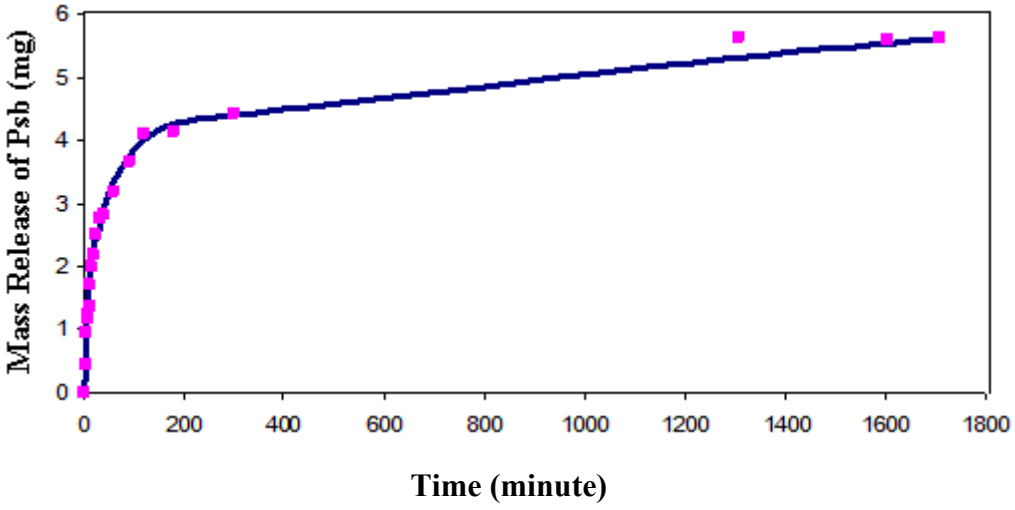


Figure 5.30. The release profile of Psb from the dense side of the single layer CA film cast with 500µm thickness and dried at 25°C. The CA content in the initial casting solution is 12.5 wt%.

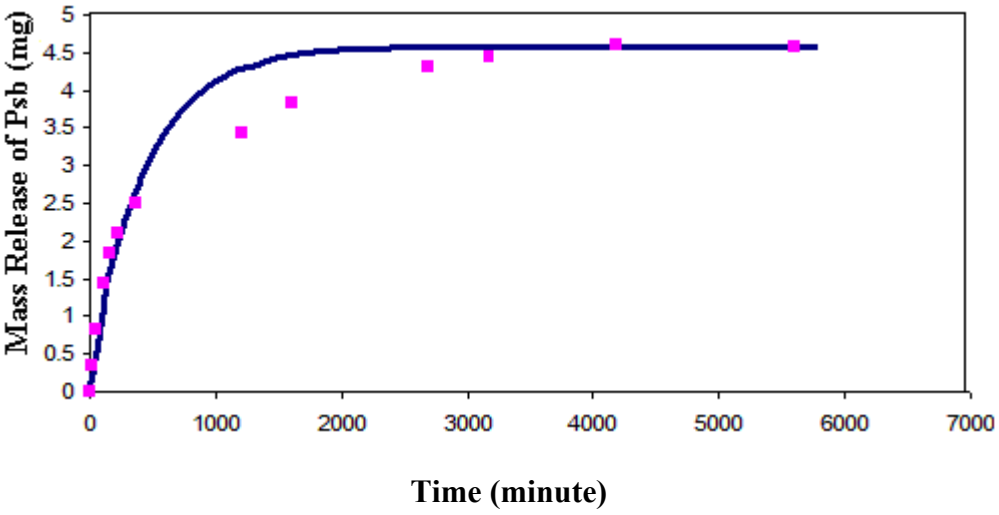


Figure 5.31. The release profile of Psb from the dense side of the multilayer CA film cast with 300µm thickness and dried at 25°C. The CA content in the initial casting solution is 12.5 wt%.

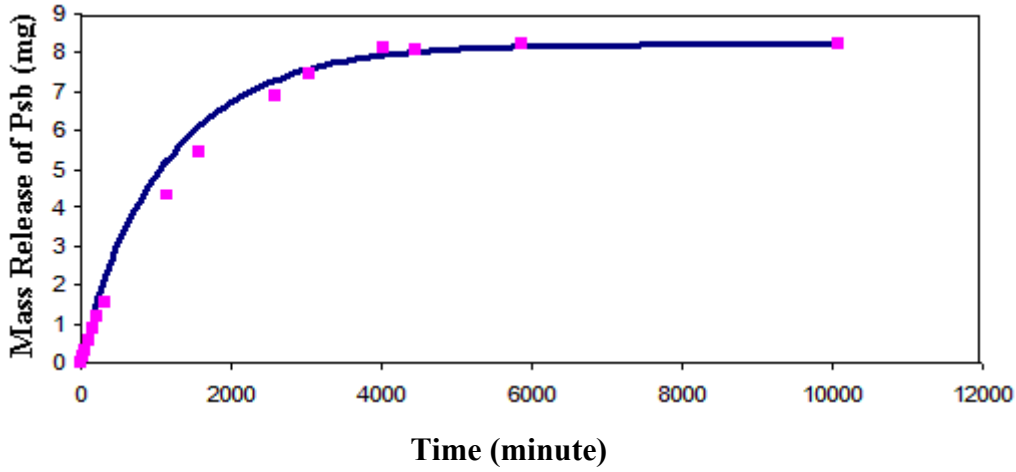


Figure 5.32. The release profile of Psb from the dense side of the multilayer CA film cast with 500µm thickness and dried at 25°C. The CA content in the initial casting solution is 12.5 wt%.

5.2.3. The Influence of Drying Temperature on the Release Rates

The release profiles of Psb from the single and multilayer films dried at 25°C and 50°C were found similar as shown by Figures 5.25, 5.33, 5.34 and 5.35 respectively.

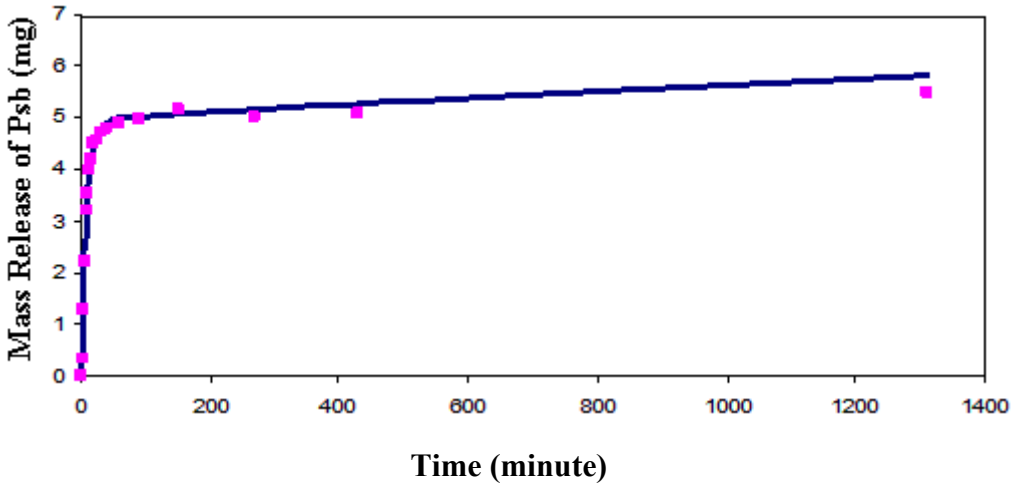


Figure 5.33. The release profile of Psb from the porous side of the single layer CA film cast with 300µm thickness and dried at 25°C. The CA content in the initial casting solution is 15 wt%.

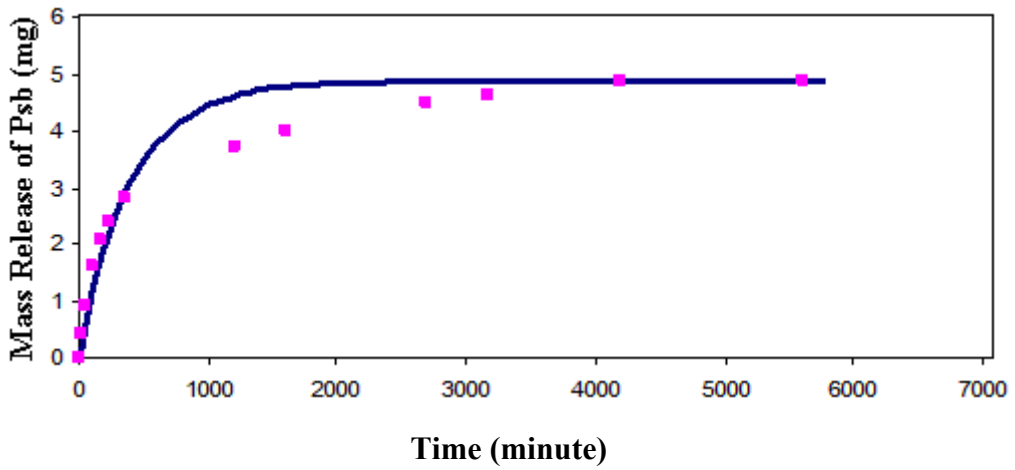


Figure 5.34. The release profile of Psb from the porous side of the multilayer CA film cast with 300µm thickness and dried at 25°C. The CA content in the initial casting solution is 15 wt%.

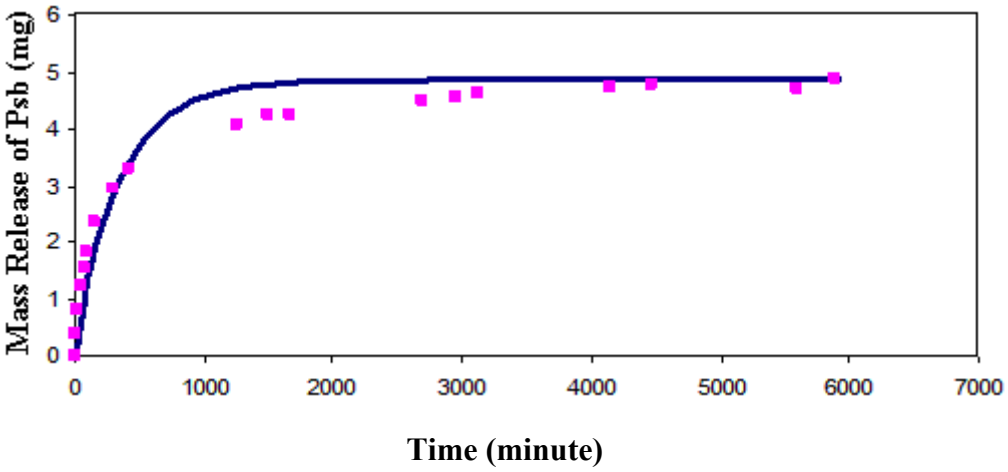


Figure 5.35. The release profile of Psb from the porous side of the multilayer CA film cast with 300µm thickness and dried at 50°C. The CA content in the initial casting solution is 15 wt%.

With increased drying temperature, the diffusion coefficients of Psb decreased slightly due to a decrease in the overall porosity and pore size of the films. The results in Table 5.3 indicate that the drying temperature does not influence the dissolution rate of crystals on the porous surfaces of the films although the crystals in the film dried at 25°C are larger. This can be explained by faster diffusion of water into this film due to its more porous structure. The crystal dissolution rates on the dense surfaces of the films dried at 25°C and 50°C were found different. The crystals dissolved at a higher rate in the film prepared with 10 wt% CA and dried at 25°C. This simply indicates that the rate of crystal dissolution in this film is controlled by the rate of penetration of water into the matrix. With increased CA content in the films, the structure becomes more dense, hence, the rate of diffusion of water into the matrix is not sufficient to initiate the crystal dissolution. Hence, the smaller crystals formed with higher drying temperature during film preparation dissolve at a faster rate as indicated by higher dissolution rate constants shown in Table 5.3.

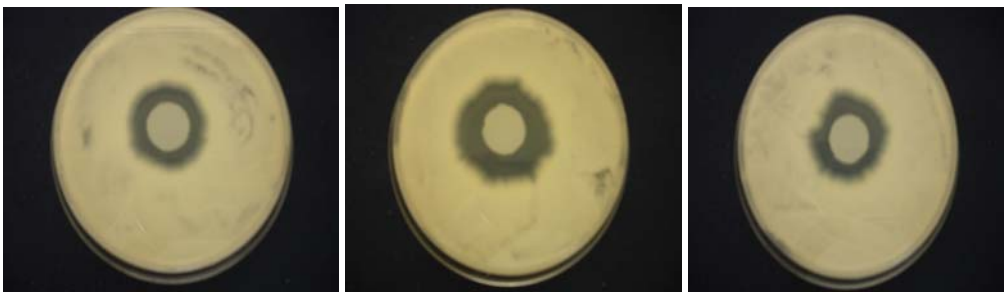
In the case of single layer films, with increased drying temperature, the partition coefficients of Psb on the porous surfaces decreased while the values obtained with dense surfaces remained almost constant. The presence of more crystals with larger sizes on the porous surfaces of the film dried at 25°C caused higher amount of Psb immobilization. An exception occurred for the films cast with 15 wt% CA in the solution. More Psb remained in the film prepared with this composition and dried at 50°C due to lower porosity and pore size of this film, hence, higher affinity to Psb to

more dense structure. The presence of a few small sized crystals present in these films does not contribute to the entrapment. In the case of multilayer films, more Psb remained in the films dried at 50°C due to lower porosity and pore size of these films.

5.3. Antimicrobial Tests

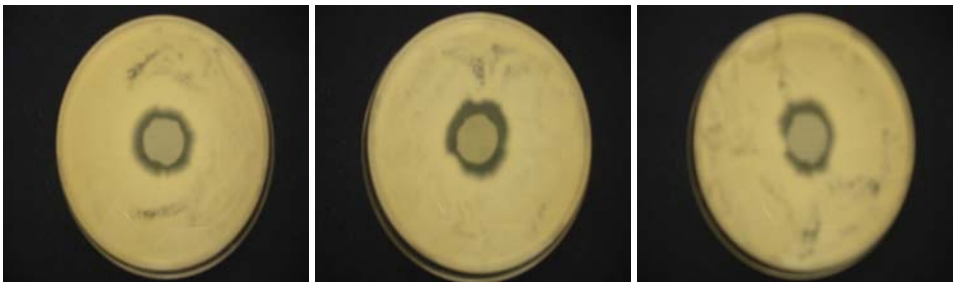
The films prepared with the highest CA content (15 wt%) were selected for antimicrobial tests since the lowest release rates were obtained from these films for each case investigated. It was thought that if sufficient antimicrobial efficiency is obtained with these films, this may indicate the antimicrobial efficiency of other films.

To test antimicrobial efficiency, *Penicillium commune* was selected as the test microorganism which is an important food-borne fungus that has been isolated from various foods such as bread, nuts, meat, and dairy products etc. and can cause the deterioration of foods. The number of spores of *P. commune* used in the tests was adjusted to $24x10^6$ spores/ml. The zone of inhibition method was used to measure the antimicrobial activity of the films. The dense surfaces of the discs were placed onto PDA agar and the clear zone formation around six sample discs are shown with the photographs in Figures 5.36 and 5.37.



a) **b)** **c)**

Figure 5.36. Antimicrobial activity of dense surfaces of single layer CA films on *P. commune*. The films were cast with a) 300µm b) 500µm c) 300µm thicknesses and dried at a) 25°C b) 25°C c) 50°C. The CA content in the initial casting solution is 15 wt%.



a) **b)** **c)**

Figure 5.37. Antimicrobial activity of dense surfaces of multilayer CA films on *P. commune*. The films were cast with a) 300µm b) 500µm c) 300µm thicknesses and dried at a) 25°C b) 25°C c) 50°C. The CA content in the initial casting solution is 15 wt%.

In Psb free control films, no clear zone formed around the disc as shown in Figure 5.38.

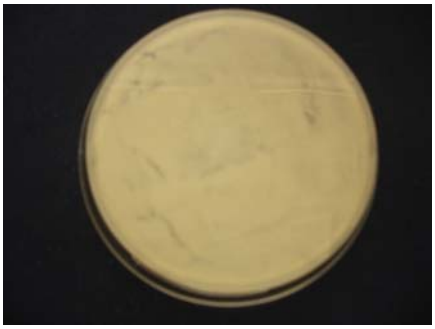


Figure 5.38. Lack of antimicrobial activity in Psb free control film.

The clear zone formation indicates the diffusion of Psb from the films into the agar medium and subsequent growth inhibition of *P. commune* in the medium.

The antimicrobial efficiency of different samples was quantified by measuring the diameter of the zones with a digital caliper. The areas of the inhibition zones were calculated based on the diameter measurement on third, fifth and seventh days are listed in Table 5.4.

Table 5.4. Areas of inhibition zones for each film.

Time (day)	Area (mm²)					
	CA1	**CA2**	**CA3**	**CA4**	**CA5**	**CA6**
DAY 3	312.8 (± 57.1) c	648.5 (±108.4) e	403.2 (±60.6) d	216 (±63.04) b	212.5 (±112.4) b	160.1 (±65.3) a
DAY 5	240.2 (±55.7) b	510.1 (±105.6) e	304.3 (±56.8) c	151.4 (±42.3) a	169.6 (±64.3) a	145.7 (±59.2) a
DAY 7	174.5 (±31.4) a	301.9 (±88.8) c	227.8 (±50.1) b	116.7 (±40.9) a	122.1 (±55.1) a	139.1 (±79) a[1]

[1]Values with different letters in the columns are significantly different (P<0.05).

The Psb amounts for each tested films were indicated in the Table 5.5.

Table 5.5. Psb amount per centimeter square of tested films.

Film Type						
	CA1	**CA2**	**CA3**	**CA4**	**CA5**	**CA6**
Psb amount in tested films (mg/cm²)	0.505	0.679	0.456	0.377	0.607	0.417

Figures 5.39 and 5.40 represent the area of clear formed zones on the fifth day for single and multilayer films, respectively.

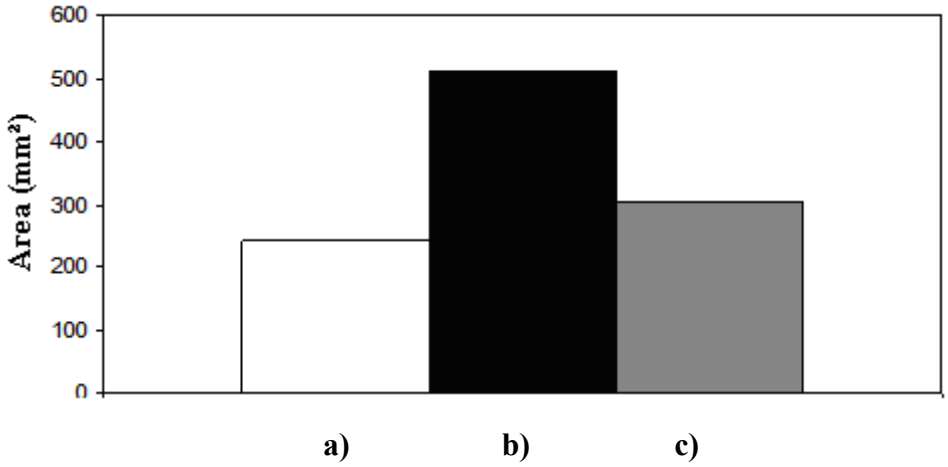


Figure 5.39. Area of formed zones on the fifth day for single layer CA films cast with a) 300µm b) 500µm c) 300µm thicknesses and dried at a) 25°C b) 25°C c) 50°C. The CA content in the initial casting solution is 15 wt%.

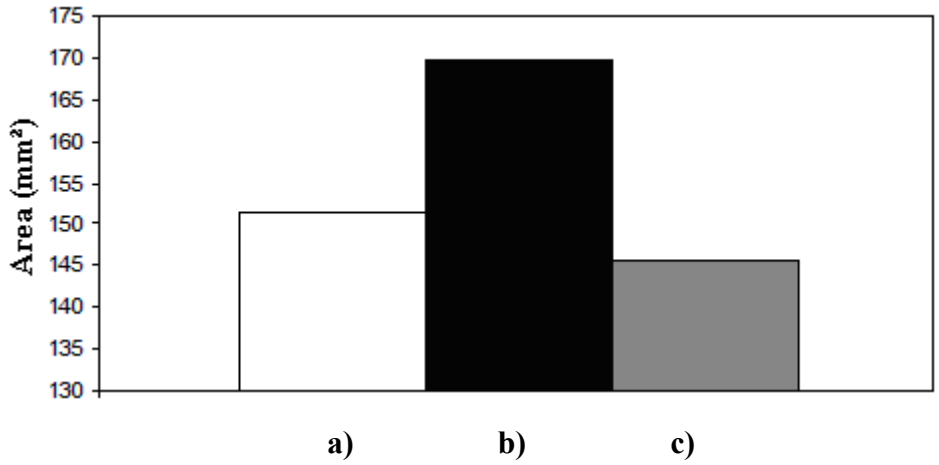


Figure 5.40. Area of formed zones on the fifth day for multilayer CA films cast with a) 300µm b) 500µm c) 300µm thicknesses and dried at a) 25°C b) 25°C c) 50°C. The CA content in the initial casting solution is 15 wt%.

For both cases, the films cast with a thicker knife (500µm) and dried at 25°C showed the highest antimicrobial activities. Smaller clear zones formed around multilayer films compared with the single layer ones due to much slower diffusion of Psb through these films. Single or multilayer films cast with a thicker knife (500µm) lead to formation of larger zones even though the diffusion coefficient of Psb decreased. This can be explained by more amount of Psb incorporated into the films with increased casting thickness. It was previously shown that increasing the drying temperature reduced the number of crystals and the size of the crystals in single layer films. Therefore, the many large crystals present on the surface of the film dried at 25°C retards the release of Psb into the agar medium. Consequently, smaller zone area was measured around these films compared with those formed around the films dried at 50°C. Antimicrobial efficiency of multilayer films dried at lower temperature (25°C) was found to be higher which can be attributed to higher diffusion rates of Psb compared with those through the films dried at higher temperature (50°C).

Figure 5.41 shows the change in the area of the zones formed with respect to time for each type of the film investigated.

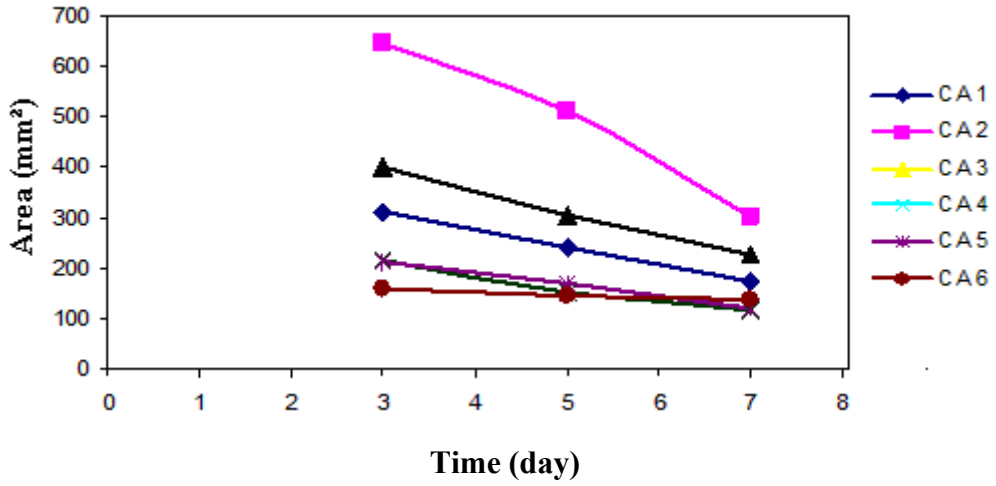


Figure 5.41. The change in the area of the zones with respect to time for each film.

Percent decrease in the area of the zones, which is due to the loss of potassium sorbate activity, was calculated based on 3rd and 7th day measurements. It was found that percent decrease in the area of the zones varies between 43% - 53% for the films denoted by CA1 through CA5. Interestingly, the change in the area of the zones formed around the film cast with 300µm thickness and dried at 50°C (the film is denoted by CA6) is almost four times smaller compared to the other cases. This may be explained by more uniform distribution of Psb in that film which allows continuous supply of Psb into the agar medium.

The reduction in antimicrobial activity of preservatives such as potassium sorbate with heat treatment was reported by Han and Floros (1998). It was found that potassium sorbate looses its antimicrobial activity exponentially with temperature and linearly with heating time (Han 1996). Even though the films prepared in this study are dried at 100°C for a period of 24 hours, they have still shown antimicrobial activity on *P. commune* spores.

CHAPTER 6

CONCLUSION

In this study, cellulose acetate based single and multilayer films including potassium sorbate as an antimicrobial agent were prepared using dry phase inversion technique. This technique allowed to prepare novel food packaging materials with structures ranging from asymmetric and highly porous to dense ones by changing the initial casting composition, wet casting thickness and drying temperature. The porous structure created with this technique initiated drying-induced crystal formation in single layer films. Psb release from these films was significantly prolonged since fast initial burst in the release rate controlled by Fickian diffusion was followed by a decrease in the rate due to slow dissolution of the crystals. It was found that the amount of Psb crystals and the size of the crystals can be varied with the overall porosity and pore size of the films. Compared with the single layer, the release rate from the multilayer film reduced and was regulated only by the diffusion of Psb since no crystal formation was observed in these films. Both single and multilayer films prepared under different conditions have shown growth inhibition on the target microorganism, *P. commune*. Compared with the multilayer films, the antimicrobial activity of single layer films was found higher due to faster release of Psb from these films.

The results of release and antimicrobial tests suggest that the films prepared in this study may be used as novel controlled release food packaging materials. However, further studies are needed to test the effectiveness of these films on selected food systems. To our knowledge, this is the first study which has shown the drying-induced Psb crystal formation in food packaging materials and the preparation of crystal dissolution-diffusion controlled release systems for food packaging applications.

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APPENDIX A

CALIBRATION CURVE

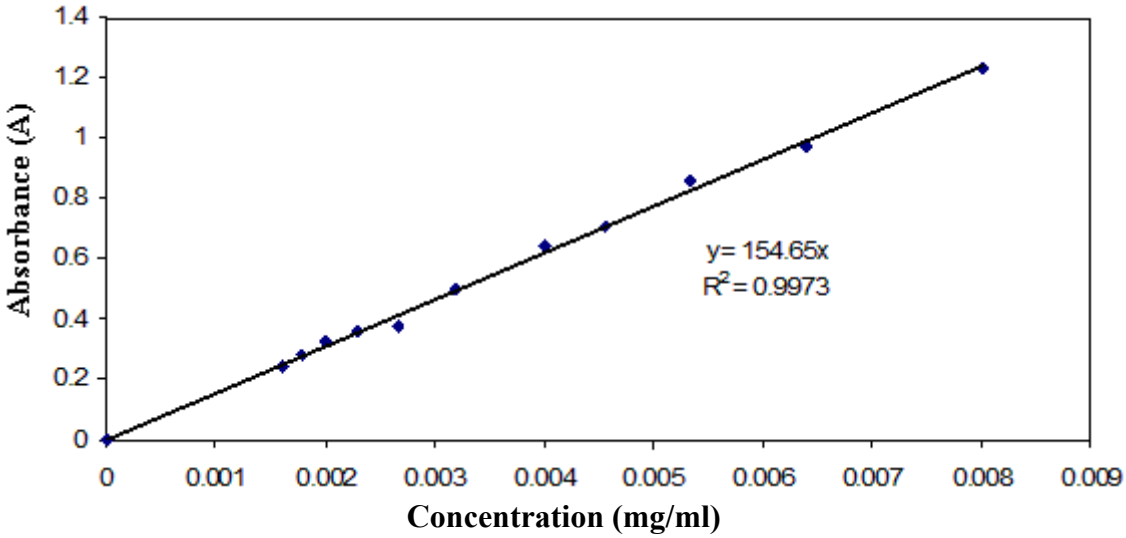

Figure A. 1. Calibration curve for sampling.