

UNIVERSITY OF GAZİANTEP
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DIFFUSIVITIES OF MOISTURE AND ANTIMICROBIAL COMPOUNDS
FROM ACTIVE PACKAGING FILMS

M. Sc. THESIS
IN
FOOD ENGINEERING

BY
SEÇİL ÖNCÜ
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**Diffusivities of Moisture and Antimicrobial Compounds from Active Packaging
Films**

M.Sc. Thesis

in

Food Engineering

University of Gaziantep

Supervisor

Prof. Dr. Sevim KAYA

by

Seçil ÖNCÜ

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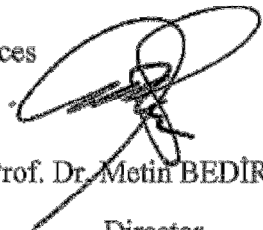
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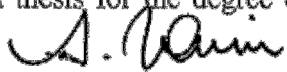
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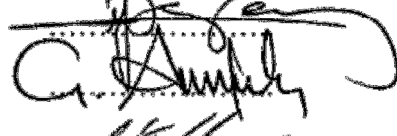

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ABSTRACT

DIFFUSIVITIES OF MOISTURE AND ANTIMICROBIAL COMPOUNDS FROM ACTIVE PACKAGING FILMS

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The release of antimicrobial agents (potassium sorbate, nisin, and chitosan) which the activity of them depends on the migration from the packaging films to the food surface and the apparent diffusivity constants (D) were examined. Moisture sorption isotherms of the active packaging films were an important parameter for the migration of the agents. In the study moisture sorption isotherms of the films were investigated and D of the films was evaluated. The release kinetics of potassium sorbate (1, 3, and 5%) and nisin (1, 2, and 3%) from polyethylene films were described using Fick's second law of diffusion and D were found to be 4.21×10^{-21} , 1.75×10^{-20} and 2.73×10^{-20} cm^2/s at 25°C ; 1.55×10^{-19} , 2.69×10^{-19} and 2.82×10^{-19} cm^2/s at 35°C , respectively. The release of chitosan from polyethylene films could not be detected. The moisture equilibrium isotherms of the films containing potassium sorbate, nisin and chitosan were determined by the Dynamic Vapor Sorption (DVS) method using the instrument, the DVS Intrinsic. Guggenheim, Anderson and De Boer (GAB) model was chosen the most suitable equation to define the adsorption isotherms of the films. The monolayer moisture content (M_0) of the films containing potassium sorbate 2%, nisin 3%, and chitosan 3% were 1.89, 0.29 and 0.09 at 20°C , respectively. D of the films between water activities of 70% to 80% RH were found as 4.12×10^{-20} , 8.35×10^{-19} and 3.23×10^{-21} cm^2/s at 20°C , respectively for potassium sorbate (2%), nisin (3%) and chitosan (3%) containing films.

Keywords: Active packaging, antimicrobial agent, diffusion, moisture sorption

ÖZET

AKTİF PAKETLEME FİLMLENERİNDEN SU VE ANTİMİKROBİYAL MADDE DİFÜZYONU

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Aktiviteleri aktif paketleme filmlerinden gıda yüzeyine geçişe bağlı olan antimikrobiyal maddelerin (potasyum sorbat, nisin ve kitosan) salınımı ve görünen difüzyon sabitleri incelenmiştir. Aktif paketleme filmlerinin su emme eşsıcaklık eğrileri antimikrobiyal madde geçişi için önemli bir parametredir. Bu çalışmada filmlerinin su emme eşsıcaklık eğrileri incelenmiş ve görünen nem difüzyon sabitleri değerlendirilmiştir. Polietilen filmlerden potasyum sorbat (% 1, 3 ve 5) ve nisin (% 1, 2 ve 3) salınımı Fick difüzyon ikinci kanunu ile izah edilmiş ve görünen difüzyon sabitleri sırasıyla 25°C de $4,21 \times 10^{-21}$, $1,75 \times 10^{-20}$ ve $2,73 \times 10^{-20}$ cm²/s olarak, 35 °C de ise $1,55 \times 10^{-19}$, $2,69 \times 10^{-19}$ ve $2,82 \times 10^{-19}$ cm²/s olarak belirlenmiştir. Polietilen filmlerden kitosan salınımı belirlenememiştir. Potasyum sorbat, nisin ve kitosan içeren antimikrobiyal paketleme filmlerinin su emme eşsıcaklık eğrileri dinamik buhar emme metodu kullanılarak dinamik buhar emme cihazı ile belirlenmiştir. Guggenheim, Anderson and De Boer (GAB) modeli su emme eş sıcaklık eğrilerini tanımlamak için en uygun eşitlik olarak seçilmiştir. % 2 potasyum sorbat, % 3 nisin ve % 3 kitosan içeren filmlerin 20 °C deki tek katman nem miktarları sırasıyla 1,89, 0,29 ve 0,09 olarak bulunmuştur. % 2 potasyum sorbat, % 3 nisin ve % 3 kitosan içeren filmlerin % 70 ve % 80 bağıl nem arası su aktivitelerindeki görünen difüzyon sabitleri 20 °C de sırasıyla $4,12 \times 10^{-20}$, $8,35 \times 10^{-19}$ ve $3,23 \times 10^{-21}$ cm²/s olarak belirlenmiştir.

Anahtar Kelimeler: Aktif paketleme, antimikrobiyal madde, difüzyon, su emme

To My Parents

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LIST OF ABBREVIATIONS

a_w	Water activity
C and K	Sorption constants
D	Apparent diffusivity constant or the apparent moisture diffusivity constant
DVS	Dynamic vapor sorption
GAB	Guggenheim, Anderson and De Boer
k	Slope of the linear regression of (M_t/M_∞) against $t^{1/2}$
l	Film thickness
LDPE	Low density polyethylene
m_o	Monolayer moisture content
M_∞	Amount of the antimicrobial agent released or the amount of diffused water at infinite time
M_t	Amount of the antimicrobial agent released or the amount of diffused water at time t
PAA	Poly (acrylic acid)
R^2	Coefficient of determination
RH	Relative humidity

CHAPTER I

INTRODUCTION

1.1 General Objective

The focus of food packaging has always been the use of proper packaging technology to minimize food losses and provide safe and sound food products.

Active packaging is a manner of packaging application that package, product, and environment affect each other to extend shelf life and enhance sensory properties while maintaining product quality (Suppakul et al., 2003). Active packaging technologies have been grown to provide better quality and safe foods as well to restrict environmental package pollution (Ozdemir and Floros, 2004; Rhim, 2007).

Antimicrobial packaging is one of many applications of active packaging (Floros et al., 1997). Antimicrobial packaging is the packaging system that is able to inhibit spoilage and pathogenic microorganisms that are causing to food contamination. Antimicrobial agents can be added into the packaging system and usage of antimicrobial polymers carry out traditional packaging requirements. The packaging system which has antimicrobial activity control microbial growth by extension of the lag period and by reduction of the growth rate of microorganisms (Han, 2000). Antimicrobial agents incorporated into the packaging system can show release, absorption and immobilization. The antimicrobial agents migrate into foods or headspace inside package to decrease microorganisms' growth at release type. Essential factors of microbial growth can be removed from the food by absorption mode. Immobilisation system does not release antimicrobial agents but eliminate microorganisms' growth at the contact surface. Surface growth of microorganisms is one of the main reasons of food spoilage in solid and semi-solid foods (Marx, 1981). Antimicrobial agents incorporated into the package material released slowly onto the food surface are developed to get over microbial growth problem

(Appendini and Hotchkiss, 2002; Han, 2000; Suppakul et al., 2003a). The inspected release of various antimicrobial agents from food packaging materials has been studied (Mastromatteo et al., 2010). There is a high customer concerns on naturally-derived antimicrobial agents due to their low health risk (Nicholson, 1998).

Development of proper antimicrobial food packaging materials depends on understanding of diffusion rate. Antimicrobial agents present in the polymer migrate onto the surface of product (Sadler and Braddock, 1991). Antimicrobial agent diffusivity provides knowledge for the estimation of antimicrobial agent release rate from the film (Han and Floros, 2000).

The measurement of moisture diffusion through packaging devices is important to develop the various food packaging solutions. Moisture sorption isotherms can be beneficial to develop applications on product stability. From a shelf life perspective, an isotherm can be used to determine what water activity and associated moisture content will inhibit growth of microorganisms and help to predict the chemical and physical stability of foods during moisture content changes. From a processing standpoint, isotherms are useful to study and control the concentration and dehydration processes, since the ease or difficulty of removing water is related to the relative vapor pressure. Isotherms are important in packaging because it helps to determine the moisture barrier properties needed in a packaging material to limit moisture gain or loss in a package (Damodaran, 2008).

The sorption isotherms can be measured gravimetrically. Dynamic Vapour Sorption instrumentation (DVS) measure the diffusion of saturated vapours through polymer packaging films (Levoguer and Booth, 2006). The sorption isotherms have been described by several mathematical models. One of the most common non linear equation to describe the sorption in food products is the GAB model (Sahin and Gülüm, 2006).

The objectives of this study were to determine:

- (a) the apparent diffusivity constant of potassium sorbate, nisin, and chitosan incorporated into polymer films,
- (b) adsorption and desorption isotherms of the films,
- (c) the constants of GAB equation and D .

CHAPTER II

LITERATURE SURVEY

2.1 Packaging

Packaging has an important role from processing and manufacturing of food through distribution, handling and storage to the final product. Food distribution would be tangled, ineffective and expensive practice without packaging. In developed countries, the packaging sector represents almost 2% of Gross National product (GNP) and nearly half of all packaging is used for food packaging (Poças, 2007).

Packaging has a many functions which are different in each other. It preserves foods from contamination and herewith provides effective distribution and storage. It makes advertising significant by facilitating brands to be created and standardized. The Codex Alimentarius Commission described the function of a food package as: 'Food is packaged to preserve its quality and freshness, add appeal to consumers and to facilitate storage and distribution' (Robertson, 2006).

The functions of packaging may be described as protection, containment, communication and convenience. Protection is an important preservation process for the packaging which is desired for fresh, frozen, dehydrated, thermal or aseptic processed foods. The containment function of packaging protects the environment from the products moving from one place to another by preventing product loss and pollution. Communication function of packaging is important at distribution, storage and consumer levels. The modern methods of consumer marketing serve by recognizing of products through branding and labeling enables supermarkets to function on a self-service. Today all packages display the Universal Product Code (UPC) that informations on the outside of food packages can be scanned easily at retail chackouts, warehouses and distribution centers. Now the application of Radio Frequency Identification (RFID) tags to the packages will revolutionize the supply

chain. Modernization and industrialization have precipitated enormous changes in life styles. Eating snack type meals rather than regular meals tend towards the designing pre-prepared easily cooked or reheated in short time products within primary package with sauces, dressings and condiments. Due to the reasons the packaging plays a significant role to meet the consumer demands for convenience (Robertson, 2006). Food packaging has progressed due to the huge demand of product safety, shelf-life extension, environmental issues, cost efficiency, and consumer convenience. The packaging systems such as innovative modified and controlled atmosphere packaging and active and intelligent packaging have developed to ensure safe and quality foods with fewer or without additives and preservatives in order to reduce food wastage, food poisoning and allergic reactions (Ahvenainen, 2000).

2.1.1 Active Packaging

Active packaging is described as deliberately addition of supplements in or on to the either packaging material or the package headspace to enhance the safety, sensory and quality aspects of foods (Robertson, 2006). Packaging usually described as active when it achieves some desired effect in the food rather than merely protecting it from the external environment (Rooney, 1995). As consumer become more health conscious and demand better quality foods the food industry has come under pressure to enhance the quality and safety of its products.

Packaging of food is essential to prevent contamination during distribution, display and handling by consumers. In addition, packages are used to contain the product, provide convenience as well as ingredient and nutrition information. Specific packaging materials are selected to protect the food product microorganisms, water, moisture, gases (oxygen), rodents, insects and light as well as to protect foods from loss of quality (carbon dioxide, moisture and aroma). Packaging technology targets have shifted from the use of conventional (passive) packaging of protecting contents to a more active application of packaging where packaging materials can absorb or emit gas to improve shelf-life or to a complete intelligent packaging system which informs the retailer or consumer about shelf-life potential. Packaging systems require that the process be functionally and cost effective, increase product safety and shelf-life, as well as be user friendly to benefit the processor, the distributor and to appeal

consumers. While in the past, packaging systems were designed to minimize the interaction between the package and its content, new packaging systems are designed to stabilize food quality over longer periods of time and this often involves deliberate interaction between the packaging material and the food inside (Rodrigues-Vieira, 2005).

Active packaging concepts as O₂ and ethylene scavengers, and CO₂ scavengers and emitters, moisture regulators, antioxidant release, release or adsorption of odours and flavours and antimicrobial packaging are briefly described.

2.1.1.1 O₂ scavenging technology

Oxidation of food compounds or spoilage by moulds in the existence of O₂ cause food deterioration. O₂ is not always removed by using modified atmosphere packaging or vacuum packaging on the other hand O₂ permeates through packaging film can not be removed by these packagings. O₂ scavenger usage provides to absorb the residual O₂ after packaging and then O₂ sensitive food quality changes can be minimized. The following concepts can be utilized by existing of O₂ scavenging technology: ascorbic acid oxidation, iron powder oxidation, enzymatic oxidation, unsaturated fatty acids such as oleic acid or linoleic acid, and immobilized yeast on a solid material (Floros et al., 1997).

2.1.1.2 Ethylene scavengers

Ethylene accumulation should be refused in the packaging to extend shelf-life and to prolong visual and organoleptic quality. Ethylene adsorbing substances are provided as sachets or integrated into films. Ethylene adsorbing technologies may add finely dispersed minerals as zeolites, clays into the packaging films (Zagory, 1995a). These incorporated minerals may adsorb ethylene and increase the film permeability thus ethylene and carbon dioxide diffuse rapidly and oxygen enter readily through the film. Ethylene scavengers do not have enough adsorbing capacity thus they are not sufficient. Each year a large quantity of fresh fruits and vegetables are lost because of physiological damage and microbial contamination (Bliidi et al., 1993).

2.1.1.3 CO₂ scavengers and emitters

Deterioration and respiration reactions produce CO₂ in foods thus the produced carbon dioxide needs to be removed from the package (Floros et al., 1997). Carbon dioxide retards microbial growth on foods such as meat and poultry and delay respiration on fruits and vegetables. Carbon dioxide has more permeability than oxygen through many packaging plastic films thus a carbon dioxide emitting system may be needed to reduce respiration rate and microbial growth.

Carbon dioxide emitters are used to prolong shelf life of foods. For instance, roasting of coffee may cause bursting of the package if the package is a can or aluminum foil pouch. Dissolved carbon dioxide in the package may cause this problem so that the usage of carbon dioxide scavenger supply to scavenge carbon dioxide from freshly roasted coffee.

2.1.1.4 Moisture regulators

Moisture can be entrapped in the packaging or consist during distribution. This kind of moisture should be removed to prevent microbial spoilage otherwise it will be absorbed by the foods or will condensate. Dry crispy products get softer, caking of milk powder and instant coffee, sweets and candies moisten because of excess water.

Removal of excess water can be made by evaporation through packaging material but the process may cause to lipid oxidation. Desiccating film or a moisture controlling sachet or pad can be used to prevent damage of excess water and to obtain desired relative humidity in the package.

2.1.1.5 Antioxidant release

Antioxidants are generally used for dried and O₂ sensitive foods to extend shelf life and improve lipids oxidation stability. Antioxidants are incorporated into plastic films and antioxidant concentrations in plastic films decrease during storage due to oxidation and diffusion of antioxidant towards the polymer surface. Antioxidative

effects of the polymer films are enhanced by addition of Vitamin E and C. Vitamin E is stable during processing and has a good solubility in polyolefins.

2.1.1.6 Release or absorption of flavors and odors

Flavor scalping is known as losing of flavor due to interaction between plastic packaging material and food flavor. Flavors are generally degraded after food processing at high temperatures or after packaging. High barrier plastic usage holds flavors in the package. Additional flavor releasing systems may be needed in case of heat seal layers of a package which have high correlation to flavors. Consumers usually smell flavors when they open the food package therefore dry instant coffee producers fill volatiles into the headspace of the package. Undesirable aromas, flavors, and odors in the package headspace can be eliminated by the usage of flavor scavengers which absorb off-flavors and off-odors.

2.1.1.7 Antimicrobial Packaging

Antimicrobial packaging is one of many applications of active packaging (Floros et al., 1997). Antimicrobial agents are added into the packaging film to prevent microbial spoilage and contamination of pathogenic microorganisms. The packaging system prevents microbial growth by extension of the lag period and reduction of the growth rate (Han, 2000). Most foods are perishable and delicate to contamination therefore antimicrobial packaging is important because of safety assurance, maintenance of quality and shelf life extension.

Antimicrobial agents in the packaging system can show release, absorption and immobilization. The antimicrobial agents migrate into foods or headspace inside package to decrease microorganisms' growth at release type. Essential factors of microbial growth can be removed from the food by absorption mode. Immobilisation system does not release antimicrobial agents but eliminate microorganisms' growth at the contact surface.

The antimicrobial agents used in the foods should follow the guidelines and regulations of the country for instance Food and Drug Administration (FDA) or

Environmental Protection Agency (EPA) in the United States. The antimicrobial agents must be approved by FDA. Various antimicrobial agents such as chemical antimicrobials, antioxidants, biotechnology products, antimicrobial polymers, natural antimicrobials and gas may be incorporated into the food packaging. The most common antimicrobial agents used in the food industry are potassium sorbate, nisin and chitosan which are preferably used in the study.

2.1.1.7.1 Potassium Sorbate

The most common antimicrobial agents used in the industry are chemical antimicrobial agents such as organic acids, fungicides, alcohols and antibiotics. Food preservatives of organic acids are sorbates, benzoic acids, sorbic acids, acetic acid, and lactic acid. Sorbic acid is a white crystalline powder. It is an unsaturated fatty acid with two double bonds and one single bond separates these two. It is called as 2, 4-Hexadienoic acid.

Potassium sorbate, calcium sorbate are its salts and incorporated into the packaging materials to prevent microbial growth in food. Potassium sorbate is white powder and soluble in water. It is used as yeast, mold, and bacterial inhibitor and a fungistatic agent in foods. It is efficient at pH 6.5 but as the pH decreases its effectivity increase. The lower the pH value of the product the lower amount of potassium sorbate is required for preservation. Han and Floros (1997) studied 1% w/w potassium sorbate containing low-density polyethylene film. They reported that potassium sorbate usage decreased the growth rate and the maximum growth of yeast and also prolonged the lag period.

2.1.1.7.2 Nisin

Nisin is a hydrophobic protein produced by *Lactococcus lactis* recommended as a natural antimicrobial agent which can be incorporated into the packaging plastic films. It is a fermentation product (Daeschul, 1989; Han, 2002) which has a strong antimicrobial effect against various bacteria. Bacteriocins are produced by the lactic acid bacteria and can be found in foods. They are non-toxic, non-antigenic in humans

and do not show negative sensory effect to foods. They have GRAS (generally recognized as safe) status for the usage in cheese, meat and vegetables. Recent studies have shown that the packaging materials incorporate nisin prevent the growth of Gram-positive bacteria such as *Brochothrix thermosphacta*, *Lactobacillus helveticus*, *Listeria monocytogenes*, *Micrococcus flavus* and *Pediococcus pentosaceus* however nisin is ineffective to moulds, yeast and Gram-negative bacteria such as *Escherichia coli*, *Vibro parahaemolyticus* and *Pseudomonas aeruginosa*. Nisin incorporated packaging materials have a restricted usage therefore incorporation of another antimicrobial agent into the packaging materials would be necessary to improve the storage stability of a variety of foods (Roseman and Daffner, 1956).

2.1.1.7.3 Chitosan

Chitosan is a natural polysaccharide and a deacetylated form of chitin. It is a natural antimicrobial for the incorporation into the packaging materials because of preventing the growth of a wide variety of fungi, yeasts and bacteria. Short or medium size chitosan instead of long chain have a quite good antimicrobial activity.

FDA has been approved chitosan as a food ingredient recently. It is a popular antimicrobial agent and the following major characteristics of chitosan make the packaging material advantageous for plenty of applications. Chitosan has a defined chemical structure. It can be chemically and enzymatically modified. It is physically and biologically functional. It can be processed into several products such as fine powders, fibers and gels. Therefore, chitosan has a great potential and becoming of a high interest. There are many research activities about chitosan and a generally accepted method for direct quantitative analysis is lacking because of chitosan must be degraded to glucosamine monomer by hydrolysis and glucosamine can be determined by chromatographic, colorimetric and fluorimetric techniques (Eikenes et al., 2005). Roseman and Daffner (1956) was studied photometric detection of acetylated glucosamine. Chitosan is soluble in various acidic solvents and it has high antimicrobial effect against Gram-positive and Gram-negative bacteria. Badawy (2012) was reported that chitosan incorporated low-density polyethylene films have shown effective microbial activity against Gram-positive and Gram-negative

bacteria. It was also reported in the article that incorporation of chitosan with nisin would give an extra antimicrobial activity.

2.1.1.7.4 Designing of an antimicrobial packaging system

Antimicrobial agents can be incorporated into a packaging material through simple blending with packaging material before extrusion (Appendini and Hotchkiss, 1976; Miller et al., 1984; Halek and Garg, 1989; Han and Floros, 1997; Nam et al., 2002). Ribbon blenders, vibratory blenders, tumble blenders, stirrer mixers, buss turbine mixers and roll mixers can be used to obtain convenient homogeneity in composition. Polymers in powder or granule form with powdered additives such as antimicrobial agents are dry blended to produce master batches in powder or granule form for batch compounding (Matthews, 1982). Continuous compounding can be achieved with soft, compact, compressed and uniformly blended components having polymers processed by a twin-screw extruder (Hess, 1978; Matthews, 1982; Cassagnau et al., 2005). After the incorporation of antimicrobial agents, the plastic film is produced by using blown extruder (Middleman, 1977).

The desing of antimicrobial packaging system necessitate controlled release technology and microbial growth kinetics. The antimicrobial agent depletes before the possible storage period if the antimicrobial agent release rate is more rapid than growth rate of target microorganisms. Therefore, the antimicrobial activity of the packaging system will be lost due to higher volume of the food than the packaging material volume and also due to the antimicrobial agent amount. While the amount of antimicrobial agents reduces, the microorganisms start to grow. When the growth of microorganisms can not be controlled because of the low release, instant growth of the microorganisms can be observed before the antimicrobial agent is released. As a result antimicrobial agent release rate from packaging to food is so important that it should be controlled and matched with the target microorganism's growth rate.

The release of antimicrobial compounds from the packaging material into the food is important mechanism to prevent the microorganisms' growth on the surface of the foods. Antimicrobial agents concentration should be provide over the minimal

inhibitory concentration on the food surface to maintain effective antimicrobial activity.

The chemical natures of antimicrobial agents are important during the designing of antimicrobial packaging system. Some antimicrobial agents are water soluble but some are not. The hydrophilic antimicrobial agents with the hydrophobic packaging plastic film will cause to the problems such as hole formation in the film, powder-blooming, loss of physical coherence and loss of transparency. The compatibleness of the antimicrobial agent and packaging material is so important. Antimicrobial agents' activity may alter according to pH. Food composition should be considered with respect to chemical nature of the antimicrobial agent. The packaging material specifications should be considered with respect to antimicrobial agent nature. Consequently this means that the pH of the packaging material depends on pH of the packaged foods (Ahvenainen, 2000).

2.1.2 Molecular Theory of Diffusion

Diffusion is thought to be the main controlling mechanism when the transfer of compounds from plastic packaging material into foods is undoubtedly a complex process. Diffusion is the mass transfer resulting from the spontaneous natural molecular movements that occur without the assistance of external forces such as shaking, mixing or even convection currents in liquids. It is thus a homogenisation, or an approach to equilibrium, brought about by random atomic or molecular motion. Diffusion process can be demonstrated experimentally by carefully placing two miscible liquids (one coloured) in contact with each other so that the minimum mixing occurs. After a time, one can see that the coloured liquid has slowly spread into the colourless component and, at equilibrium, will be evenly dispersed throughout the whole vessel. This process occurs most rapidly in gases, only slowly in liquids and infinitesimally slowly in the case of two solids in contact. There is thus a natural tendency for materials to diffuse from high concentration to low concentration areas until at equilibrium there will be no concentration gradient. More correctly it is usual to state that diffusion takes place from high chemical potential into low chemical potential areas until an overall constant chemical potential is attained.

In an analogous fashion to changes in concentration, where differences in temperature exist across a boundary, heat will flow from a point of higher temperature to one of lower temperature. Similar changes take place where pressure differentials exist. Chemical potential is, therefore, a measure of the tendency of a component to migrate through the concentration gradient.

The transference of organic molecules from the packaging material into the food is a complex phenomenon. Most of mathematical treatments of transport processes are derived initially from a gaseous diffusion consideration, where the molecules perform random motions following the concepts of the kinetic theory of gases. Thus, diffusion is a spreading out and can be observed to take place against both gravity and through porous barriers. In liquids, the cohesive forces are much stronger and the molecules are closer together than for gases so that approximately 95 per cent of the space in a liquid is occupied by the molecules themselves. There is still considerable freedom for motion, although diffusion coefficients in liquids are of the order of 10^{-5} cm²/s only, meanwhile one million times slower than in gases (Ahvenainen, 2000). In solids, most molecules are fixed in a crystalline regular lattice, except at the surface where imperfections may occur. Diffusion coefficients in solids are much lower still-around 10^{-11} cm²/s (Ahvenainen, 2000).

The diffusivity antimicrobial agents considering from one side of the films into solutions and the diffusivity of water through the films was determined by Fick's second law, as follows (Crank, 1975):

$$\frac{\partial C_a}{\partial t} = D(t) \frac{\partial^2 C_a}{\partial x^2}, \quad (2.1)$$

Where C is the concentration of antimicrobial agent, D is the apparent diffusivity constant, x is the spaces coordinate in the direction of diffusion; and t is time.

Here an auxiliary variable T is introduced (Crank, 1975):

$$\frac{dT}{dt} = \frac{D(t)}{l^2} \quad (2.2)$$

Where l is the film thickness. After integrating Equation (2.2), the relationship between T and t becomes:

$$T = \int_0^t \left[\frac{D(t)}{l^2} \right] dt \quad (2.3)$$

Then by utilizing the chain rule and by substituting into Equation (2.1) the linear differential equation is obtained:

$$\frac{\partial C}{\partial T} = l^2 \frac{\partial^2 C}{\partial x^2} \quad (2.4)$$

Equation (2.4) can be solved for the following initial boundary conditions:

$$C = C_0, \quad 0 < x < l, \quad T = 0$$

$$C = 0, \quad x = 0, \quad T > 0$$

$$C = 0, \quad x = l, \quad T > 0$$

Where C_0 is the initial antimicrobial agent concentration in the film.

The solution of Equation (2.4) under these conditions is:

$$C = \frac{4 C_0}{\pi} \sum_{m=0}^{\infty} \frac{1}{(2m+1)} \sin \frac{(2m+1)\pi x}{l} \exp[-(2m+1)^2 \pi^2 T] \quad (2.5)$$

The antimicrobial agents release from the films can be calculated by integrating Equation (2.5) over space and time:

$$\frac{M_t}{M_{\infty}} = 1 - \frac{8}{\pi^2} \sum_{m=0}^{\infty} \frac{1}{(2m+1)^2} \exp[-(2m+1)^2 \pi^2 T] \quad (2.6)$$

Where M_t is the amount of the antimicrobial agent released or the amount of water diffused at time t , and M_{∞} is the amount of the antimicrobial agent released or the amount of water diffused at infinite time.

Intergrating Equation (2.5) over the film thickness to obtain the release as a function of time yields:

$$\frac{M_t}{M_{\infty}} = 1 - \frac{8}{\pi^2} \sum_{m=0}^{\infty} \frac{1}{(2m+1)^2} \exp\left[-\frac{(2m+1)^2 \pi^2 D t}{l^2}\right] \quad (2.7)$$

Where l is the film thickness. Equation (2.7) was used to simulate the theoretical diffusion curve. M_t/M_{∞} were calculated and plotted as a function of square root of time. $M_t/M_{\infty} < 2/3$ of diffusion processes (Crank, 1975; Sfirakis & Rogers, 1980;

Mohney, Hernandez, Giacin, Harte, & Miltz, 1988; Redl et al., 1996; Bichara, Fugit, & Taverdet, 1999) was used to calculate the apparent diffusivity constant:

$$D = \left(\frac{kl}{4}\right)^2 \pi \quad (2.8)$$

Where k is the slope of the linear regression of (M_t/M_∞) against $t^{1/2}$.

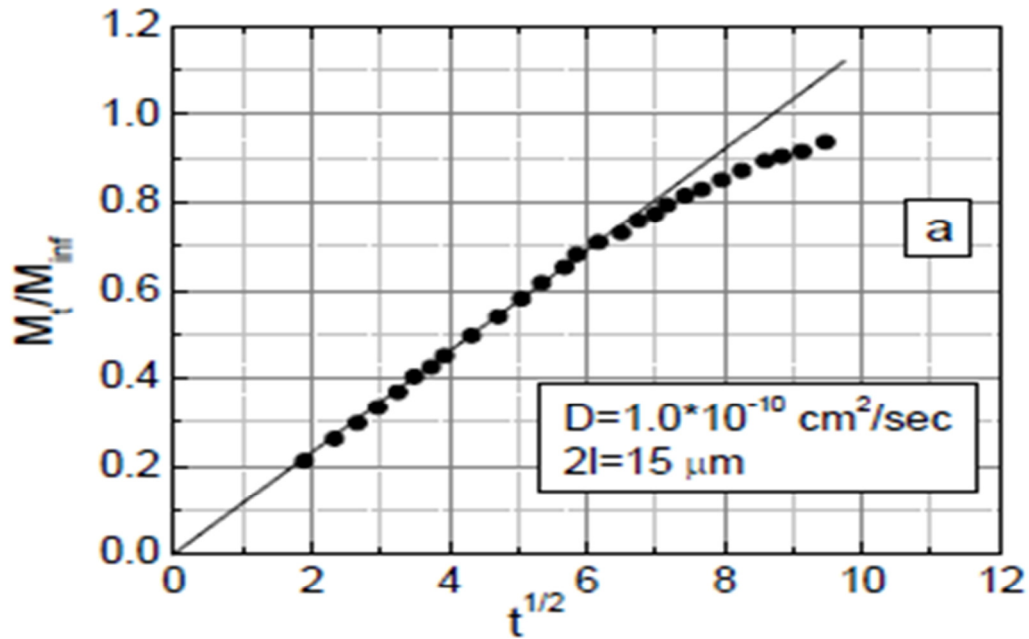


Figure 2.1 Release of antimicrobial agent as a function of time; data were extracted from literature (Fieldson and Barbari, 1993)

Garlanda and Masoero (1996) applied the diffusion theory to the migration of constituents from plastics into solvents, to determine the effect on migration of parameters such as time, temperature, polymer thickness, etc. They considered the general solution of Fick's equation assuming D is constant, i.e. no penetration of the polymer by the food. This treatment gives an average concentration of a constituent C . Thus, after the initial stages, the linear equation with respect to square root of t is replaced by a line curving rapidly towards the x-axis until it reaches an equilibrium value. This is the shape of curve observed in practice. Assuming D equal to 10^{-12} cm^2/s and films of thickness 10, 50 and 100 μm , it was possible to relate migration with time for different thickness of polymer and to compute different equilibrium levels. Thus, the equilibrium value of migration is a function of the initial

concentration in the polymer. The time to reach this limiting value varies with the coefficient of diffusion and the thickness of the sample.

Davies (1974) investigated the migration of styrene monomer into foods such as orange squash, margarine and single and double cream. Starting with Fick's diffusion equation, a solution of the equation was adapted derived by Crank, for the situation in which a solid sheet of polymer is in contact with a stirred solution. An equation was obtained and in the equation M_t was the amount of styrene which has migrated at time t and M_∞ was the amount of styrene which has migrated at equilibrium. The theoretical treatment was evaluated by using a curve fitting technique, to determine how well the experimental data coincided with a series of calculated curves, in which unknown constants were varied systematically. In the case of aqueous systems the fit was very good.

Robinson and Becker (1965) calculated D of oil entering a polyethylene film using an equation derived from the Fick's laws. The value of D was found to be $26 \times 10^{-10} \text{ cm}^2/\text{s}$ at 30°C .

2.1.3 Theory of Dynamic Vapour Sorption

The moisture sorption isotherms are important for the development of food packaging solutions. Moisture loss of the packaging systems during the storage periods must be minimized to extend shelf life of the foods. Most foods experience physical, chemical, and microbiological changes over time and are therefore non-equilibrium systems occur (Schmidt 2004). However, the concept of water activity (a_w) has proven to be an extremely useful and practical tool in the food industry and in food science research. The most usable aspects of the water activity for food systems and ingredients are the relationship of moisture content as a function of relative humidity at the same temperature. This relationship is unique for each food material and is known as the moisture sorption isotherm. The isotherm describes the amount of water sorbed (adsorption or desorption) at equilibrium by a material at a known a_w at a constant temperature (Bell and Labuza, 2000).

As mentioned, all food materials have their own characteristic moisture sorption isotherm. However, most sorption isotherms have a sigmoidal shape. From the

practical view of obtaining the moisture sorption isotherm of a food system, there are two approaches: adsorption and desorption. For an adsorption isotherm, samples need to be dried to near zero percent moisture. The sample is then hydrated with water in order to form the moisture sorption isotherm. For a desorption isotherm, samples are hydrated completely first and then moisture is removed to obtain the isotherm. One sample has moisture removed from the natural state (desorption curve), while a different sample has moisture added to the natural state (adsorption curve). Isotherm data points (moisture content and water activity) are determined by measuring equilibrium moisture contents at several water activities (Bell and Labuza, 2000).

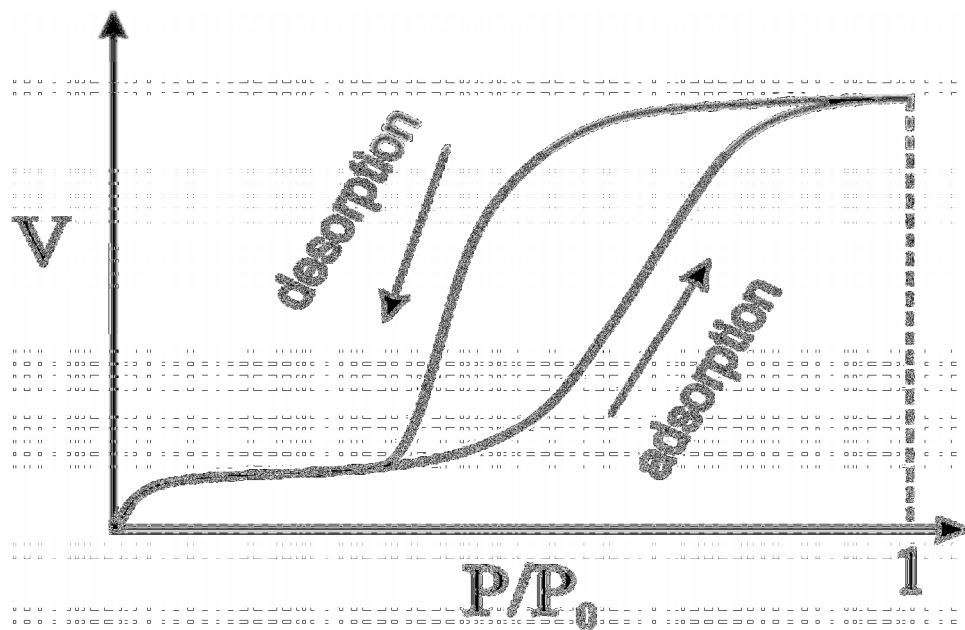


Figure 2.2 Adsorption and desorption isotherms, data were extracted from literature: (Shamiryan, 2012)

As shown in Figure 2.2, an adsorption or desorption process can form the moisture sorption isotherm curve. The difference in moisture content between the adsorption and desorption curves for a given water activity value is due to hysteresis. One reason for this difference is some solutes may be supersaturated below their

crystallization a_w during drying (desorption) and therefore hold more water as a_w is lowered. Also, upon desorption capillaries can empty differently. The surface tension and wetting angle show difference between adsorption and desorption, for desorption resulting in a higher moisture content (Labuza and Altunakar, 2007).

Moisture sorption isotherms can be good for several processing and product stability applications. From a shelf life perspective, an isotherm can be used to determine what a_w and associated moisture content will inhibit growth of microorganisms and help predict the chemical and physical stability of foods in their moisture content changes. In terms of product development, an isotherm helps in formulating food mixtures to avoid moisture transfer among ingredients and permit for ingredient selection to change the a_w . From a processing standpoint, isotherms are useful to study and control the concentration and dehydration processes, since the ease or difficulty of removing water is related to the relative vapor pressure. Isotherms are important in packaging because it helps determine the moisture barrier properties needed in a packaging material to limit moisture gain or loss in a package (Damodaran, 2008). The sorption isotherms can be measured by three different measurement techniques such as manometric, gravimetric or hygrometric (Chirife, 1978). The sample weight is measured with a balance in the gravimetric methods. When the vapour pressure of water is at equilibrium with a sample of given moisture content, the vapour pressure of water is measured by the manometric methods. The equilibrium relative humidity with a sample of given moisture content is measured by the hygometric methods (Mathlouthi and Roge, 2003).

A novel method for rapid measurement of the saturated vapours diffusion through the polymer packaging system determined by using Dynamic Vapour Sorption instrumentation (DVS) (Levoguer and Booth, 2006). The DVS Intrinsic is the latest gravimetric water sorption analyzer. It is a fully automated humidity generating instrument that is controlled from a computer program and utilizes the same principle as the DVS advantage. However, this instrument was specifically designed to meet the needs of small to mid-size laboratories and plants. There are a number of advantages of these humidity generating instruments over the traditional saturated salt slurry method. The equilibration time is about 10 to 100 times faster than the standard method. The instruments are fully automated and use a computer interface which removes the labor intensive nature and required supervision of the salt slurries.

The instruments maintain the relative humidity and temperature conditions of the material during the experiment. Due to the high precision and sensitivity, sample sizes can be small (Levoguer and Williams, 1997).

The correlation between the water activity and moisture content mathematically expressed and to describe the sorption isotherms several models such as nonlinear, linear and regressional have been developed. Sorption isotherms can be approached to linear equations in some ranges of a_w (Yan et al., 2008). Some models described adsorption by developing with a theoretical basis (Rockland and Stewart, 1981). The most common equations used to describe the moisture sorption isotherms are the GAB model, the Langmuir equation, the BET equation, the Peleg model and (Sahin and Gülüm, 2006).

The GAB model was used in the study. The meaning of the GAB is Guggenheim, Anderson and De Boer who derived the equation in 1966, finally. This model is the refinement of Langmuir and BET theories thus has many advantages over the other models.

The GAB model is expressed as in Equation (2.9):

$$m_w = \frac{m_0 C K a_w}{(1 - K a_w)(1 - K a_w + C K a_w)} \quad (2.9)$$

Where m_0 is the monolayer moisture content; C and K are the adsorption constants. When K is 1, the GAB model becomes the BET equation. The GAB model contains two additive terms. The first term describes the mono-molecular layer expression in Langmuir's adsorption isotherms. The second term describes the Raoult's law's multilayer adsorption (Lomauro et al., 1985).

It is represented in Equation (2.10):

$$\frac{m_w}{m_0} = \frac{(C-1)K a_w}{(1 - K a_w + C K a_w)} + \frac{K a_w}{(1 - K a_w)} \quad (2.10)$$

The GAB model describes behavior of the sorption in a wide range of a_w (0 to 0.9) therefore it is generally used to analyze fruits, meat and vegetables. The European COST 90 project recommends usage of the GAB equation (Gaoula et al., 2008). The moisture content values at higher water activity levels ($a_w > 0.93$) are underestimated

by the GAB model that the model is unsuitable for high humidity ranges (Basu et al., 2006).

CHAPTER III

MATERIAL & METHOD

3.1 Materials

Active packaging films containing different amount of antimicrobial compounds potassium sorbate (1-5%), nisin (1-3%) and chitosan (1-3%) were supplied by Naksan Plastik ve Enerji Sanayi ve Ticaret A.Ş (Gaziantep). The films having different structures were both used for the determination of the diffusivity of antimicrobial agents and moisture. Potassium sorbate (1, 3 and 5%) incorporated polyethylene films were single layer with 40 µm thicknesses and nisin (1, 2 and 3%) incorporated polyethylene films with 70 µm thicknesses were multi layer films formed with polyethylene (with antimicrobial agent)/polyamide/polyethylene. These active films were used in the diffusivity experiments of antimicrobial agents through the films. On the other hand 70 µm multilayer films containing nisin (1-3%), chitosan (1-3%) and potassium sorbate (1-2%) were used for the moisture diffusion experiments. The concentrations and the name of the agents of the films used to define the type of the films throughout the study. All the chemicals used in the experiments were analytical grade. High pressure liquid chromatography (HPLC) grade water was used throughout the study.

3.2 Methods

3.2.1 Determination of Potassium Sorbate Release

The diffusivity of antimicrobial compounds from the films can be determined using a technique offered by Ozdemir and Floros (2001). Films containing potassium sorbate (1-5%) were cut a square with dimensions of 2 cm x 2 cm using a

sharp razor knife. Each antimicrobial compound containing film having weight of approximately 0.1 g was immersed into a beaker containing 50-50% of water-glycerol solution. The beaker was placed in a controlled room temperature at $25^{\circ}\text{C}\pm 2^{\circ}\text{C}$. The solutions were well-stirred with an incubator shaker (Innova 40R, New Brunswick Scientific, New Jersey, USA) at 950 rpm 4 mL of samples of solution were removed from the beaker into test tubes at certain intervals were kept in refrigerator until measurement.

The absorbance values for the standards and the samples were measured at 254 nm using a UV/VIS spectrophotometer (Lambda 25, Perkin Elmer, Shelton, CT, USA). The amounts of potassium sorbate in the samples were evaluated using the standart curve.

3.2.2 Determination of Nisin Release

Nisin, being a hydrophobic protein can be determined by using the principle behind the Lowry method. Protein by Folin Reaction (Lowry et al., 1951) has been the most commonly used method to estimate the amount of proteins already in solution or easily-soluble in dilute alkali. Bovine serum albumin (BSA) stock solution was prepared and kept for 1 hour before starting the experiment. Concentration of the BSA stock sol'n was 0.1 mg/mL. The Lowry solution is a mixture of solution A, B and C except the Phenol Reagent. Solution A was prepared with 2% Na_2CO_3 in 0.1 N NaOH for 100 mL, solution B was prepared with 1% NaK Tartrate in H_2O for 10 mL and solution C was prepared with 0.5% $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ in H_2O for 10 mL. Finally the Lowry solution was the mixture of solution A, B and C with a ratio (v:v) of 48:1:1. Phenol Reagent was prepared as 1 part Folin-Phenol [2N]:1 part H_2O .

Nisin containing (1-3%) multi-layer square films (approximately 0.1 g) of dimensions 2 cm x 2 cm were cut using a sharp razor knife. Each film was immersed into a beaker containing 50 ml of HPLC grade water. The beaker was placed in a controlled temperature at $35^{\circ}\text{C}\pm 2^{\circ}\text{C}$. The solution was well-stirred with a shaking incubator at 900 rpm. 4 mL of samples of solution were removed from the beaker into test tubes at certain intervals were kept in refrigerator until measurement.

The test tubes were set up in rack and for all samples duplicate tests were run. (0, 0.2, 0.4, 0.6 and 0.8 mL) BSA were added to the tubes and diluted to 1 mL HPLC grade water. 1 mL of sample solutions containing nisin was transferred to the tubes. 2 mL of Lowry solution was added to each test tube and incubated at room temperature for 10 min. Then 0.2 mL of dilute Folin-phenol reagent was added. A vortex (VX-200, Labnet International Inc., Edison, NJ, USA) was used to mix each tube immediately. All samples were incubated once more for 30 minute at room temperature. The end product of the Folin reaction has blue color. The absorbance values for the standards and the samples were determined at 550 nm. The amount of protein in the sample was estimated via reading the absorbance of the end product of the Folin Reaction against a standard curve of the BSA solution.

3.2.3 Evaluation of Dynamic Vapour Sorption

Multi-layer packaging materials containing potassium sorbate (1-2%), nisin (1-3%) and chitosan (1-3%) were used and the film thickness was 70 μm . The moisture sorption isotherms of the polymer films were measured at 20°C by the Dynamic Vapor Sorption (DVS) instrument (DVS Intrinsic, Surface Management Systems, London, UK). The sample was exposed to increasing steps in humidity from 0% RH to 90% RH with 10% increments and back down to 0% RH, such that both sorption and desorption steps were measured for each discrete humidity. The dry nitrogen flow rate used was 200 scc and the dm/dt was set at 0.0010%/minute. The minimum stage time (minimum experiment time at each relative humidity) was 180 minutes and the maximum stage time (maximum experiment time at each relative humidity) was 600 minutes depending on preliminary studies. The experimental results were used to plot the curve of the equilibrium moisture content against water activity.

The GAB model was chosen to fit the adsorption isotherm of polymer films over a large range of water activities (0 to 90% RH). The accuracy of the fitting was evaluated with respect to coefficient of correlation (R^2). D was calculated using Equation (2.8) representing Fick's second law of diffusion from sorption isotherms of the films at a water activity range from 70 to 80% RH.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 General

The diffusivities of the antimicrobial agents and moisture were examined and the diffusivity constants of them were evaluated. Adsorption and desorption isotherms of the antimicrobial agents incorporated into polymer films were performed by using the DVS Intrinsic instrument. The GAB model was applied to fit the adsorption isotherms of polymer films and the apparent diffusivity values of water of the films were evaluated from the isotherm. At the each experiment, two replicates of each film were tested and the mean were reported.

4.2 Potassium Sorbate

In the study the potassium sorbate release was experimented by addition of glycerol to water. In this way water activity of the medium was decreased and a food matrix simulant system was obtained. Glycerol was used as a humectant additive that binds water and control a_w . Ledward (1985) discussed intermediate moisture of meats development and concluded that a water activity less than 0.85 could be achieved using glycerol as 15 to 45% of the food product.

The standart curve of the potassium sorbate solution represented in Figure 4.1 was used to determine the concentration of potassium sorbate released into the solution. The evaluated potassium sorbate released amounts (M_t) at time t was determined by using the Figure 4.1. The released potassium sorbate amount was divided with (M_∞) values. In this study (M_∞) values assumed as the initial amount of potassium sorbate in the polymer films. (M_t/M_∞) against $t^{1/2}$ was plotted to determine the k constant which was used in Equation (2.8). Figure 4.2 shows that plot of (M_t/M_∞) versus $t^{1/2}$ were linear for the different potassium sorbate containing packaging films. The slope

of the linear regression gave k constant that would be used for determining the apparent diffusivity constants.

D was calculated in this study with the early stage method of Equation (2.8). The diffusivity constants and coefficient of determination were summarized in Table 4.1.

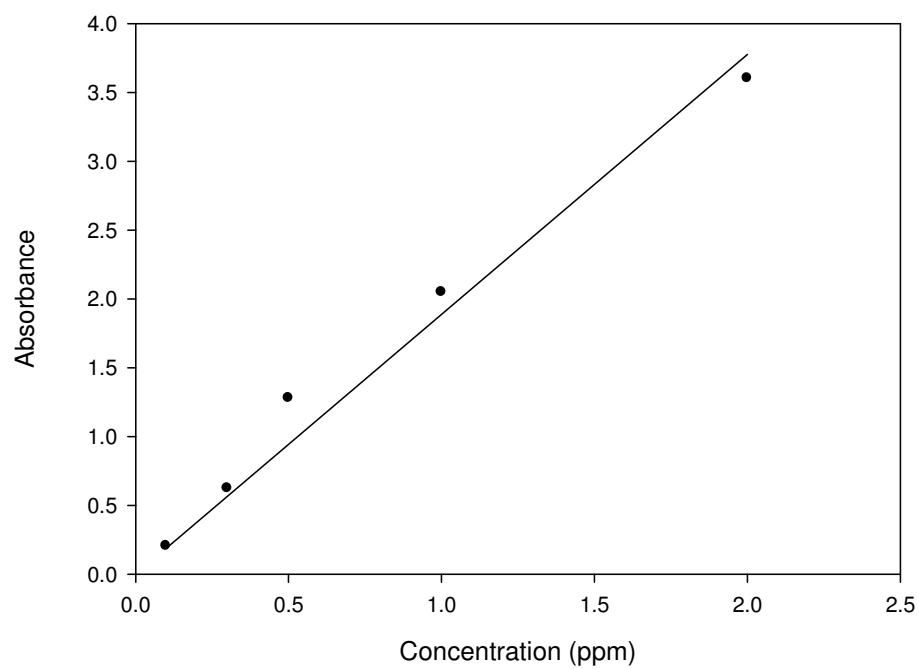


Figure 4.1 The standart curve of potassium sorbate solutions having different concentrations.

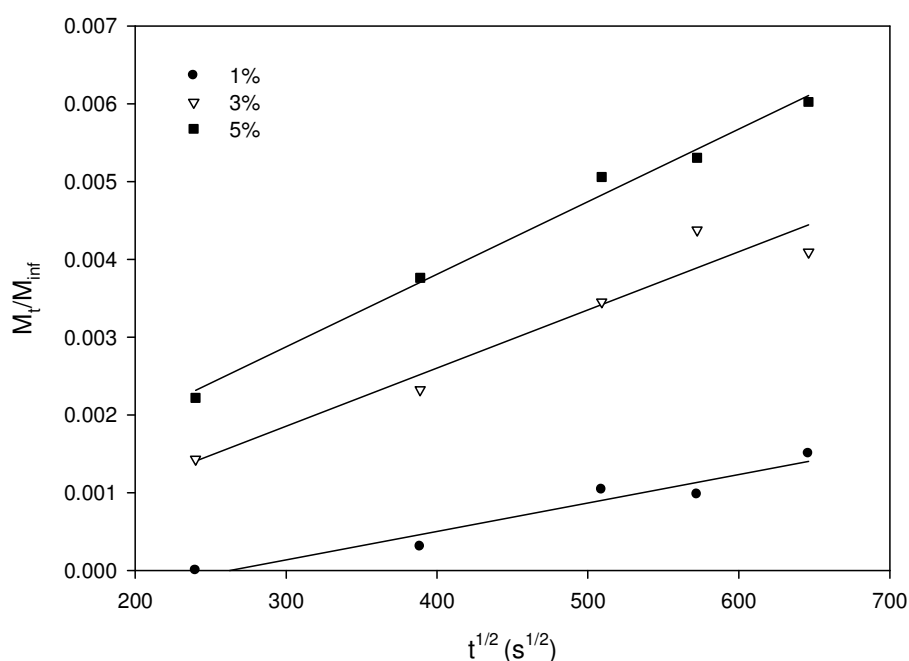


Figure 4.2 Release of potassium sorbate from antimicrobial polymer films.

Table 4.1 The apparent diffusivity constants of potassium sorbate and coefficient of determination

Potassium sorbate concentration	$D (cm^2/s)$	R^2
1%	4.21×10^{-21}	0.94
3%	1.75×10^{-20}	0.93
5%	2.73×10^{-20}	0.99

Table 4.1 shows that the apparent diffusivity constants increased with increasing initial potassium sorbate concentration in the films. There are a few studies evaluating the release of potassium sorbate from the polymer films and determining the apparent diffusivity constants of potassium sorbate.

Han and Floros (1997) reported that the potassium sorbate apparent diffusivity constant from low density polyethylene (LDPE) was $1.83 \times 10^{-8} cm^2/s$ at $25^\circ C$. They studied movement of the antimicrobial substance from high concentration area

(packaging material) to low concentration area (food) to prevent microbial spoilage in case of the microorganism-controlling material as antimicrobial packaging material. The release of the antimicrobial substance through plastic films predicted with lag time method which was completely different method from our method of study. It had been reported in their study that potassium sorbate had the highest diffusivity through LDPE compared to other films tested such as high density polyethylene (HDPE), polypropylene (PP) and biaxially oriented polyethylene terephthalate (BOPET) films. Therefore, they suggested that LDPE was a good material for the layer containing active substance or fast diffusion control layer. However, the apparent diffusivity constant values were determined in the study was lower than the determined value in the mentioned article. The reasons may be first the differences in the concentrations applied in the both experiments and the possible entrapment of the potassium sorbate within the polymer matrix. Increasing concentrations improves the diffusion phenomena (Figure 4.2). The concentrations of the potassium sorbate studied were in the range of 1-5% but higher concentration (20% w/v) had been applied in the article thus the release of potassium sorbate from the films were higher than in this study.

Ozdemir (1999) reported that the diffusivities of potassium sorbate at 25°C ranged from 5.38 to 9.76×10^{-11} m²/s. The diffusion of potassium sorbate from whey protein films was studied. Compared to synthetic polymers, such as LDPE, potassium sorbate diffusion coefficients in whey protein films were higher. Furthermore, Ebnesajjad (2013) reported that the release of the antimicrobial agents from hydrophobic plastic is low. Therefore the low diffusion of potassium sorbate may be due to its hydrophilic nature compared with the hydrophobic packaging plastic film.

4.3 Nisin

Nisin release through the active films were studied without glycerol due to the lack of glycerol solubility in Lowry solutions.

The standard curve of the BSA solution represented in Figure 4.3 was used to determine the concentration of nisin released into the solution. The evaluated nisin released amounts (M_t) at time t was determined by using the Figure 4.3. The

determined released nisin amounts divided with (M_∞) values. In this study (M_∞) values assumed as the initial amount of nisin in the polymer films. (M_t/M_∞) versus $t^{1/2}$ was plotted to determine the k constant which was used in Equation (2.8). Figure 4.4 shows that plot of (M_t/M_∞) versus $t^{1/2}$ were linear for the both three of the different nisin containing packaging films. The slope of the linear regression gave k constant that would be used for determining the apparent diffusivity constants.

D was calculated in this study with the early stage method Equation (2.8). The apparent diffusivity constants and coefficient of determination were summarized in Table 4.2.

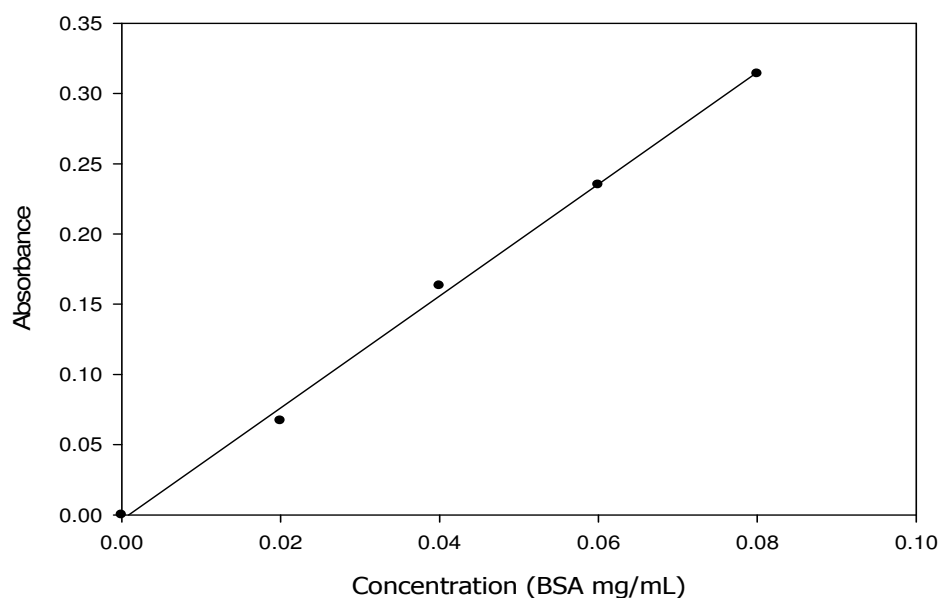


Figure 4.3 The standard curve of the BSA solution (BSA mg/mL).

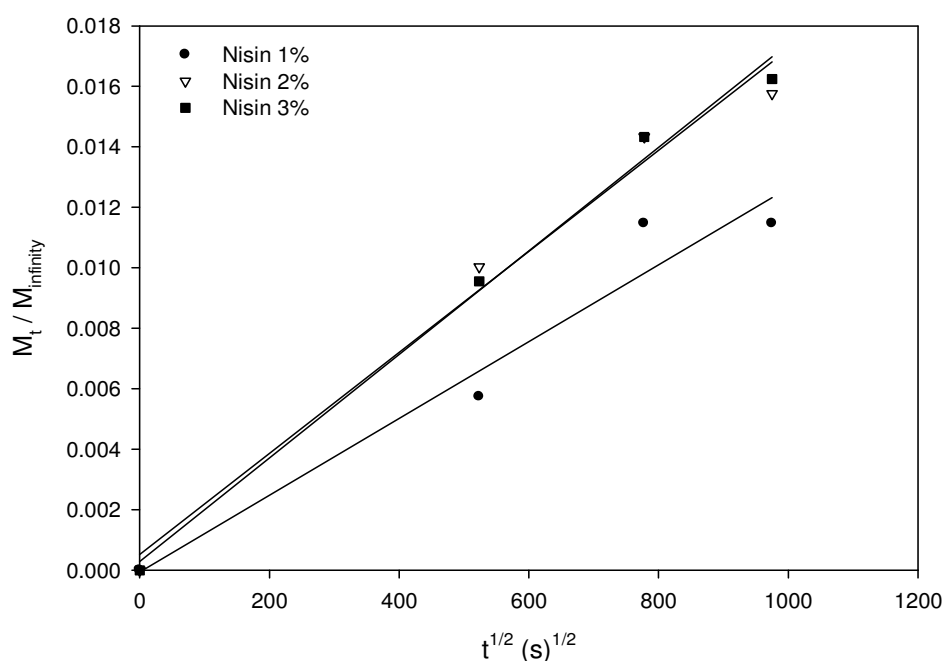


Figure 4.4 Release of nisin from antimicrobial polymer films.

Table 4.2 The apparent diffusivity constants of nisin and coefficient of determination

Nisin concentration	D (cm ² /s)	R^2
1%	1.55×10^{-19}	0.95
2%	2.69×10^{-19}	0.98
3%	2.82×10^{-19}	0.99

Table 4.2 shows that D increased with increasing initial nisin concentration in the films. There are a few studies evaluating the release of nisin from the polymer films and determining the apparent diffusivity constants of nisin. Bastarrachea et al. (2010) reported that the apparent diffusivity constants of nisin as 0.93×10^{-10} , 2.29×10^{-10} and 5.78×10^{-10} cm²/s at 5.6, 22 and 40°C, respectively. They studied the release kinetics of nisin from poly(butylene adipate-co-terephthalate) film and used Fick's second law of diffusion to determine the apparent diffusivity constants.

The apparent diffusivity constants determined in the study were lower than the constants which were determined in the study above. The low apparent diffusivity constants could be due to many reasons as explained below:

Nisin is a hydrophobic protein (Klaenhammer, 1993) and the nature of polymer films was also hydrophobic. The releasing agent and the polymer used as a packaging material are in the same chemical group so that they will show same affinity and will interact with each other leading to a high retention of the releasing agent in the polymer matrix (Franz, 2000). Furthermore, Joosten and Nunez (1995) reported that nisin has tendency to aggregate, to form large complexes, and to adhere to hydrophobic surfaces due to its hydrophobicity.

On the other hand, the antimicrobial film used in the study was sandwiched in a multilayer structure. Han and Floros (1998) reported that the multilayer film structure reduce the release of the active component by interposing barriers.

4.4 Chitosan

The multi-layer active packaging films incorporated chitosan (1-3%) having thicknesses of 70 μm were experimented to find out the diffusivity of chitosan through the films using spectrophotometric and HPLC (HPLC 1200, Agilent Technologies, Palo Alto, CA, USA) techniques. Quantitative determination of chitosan into the HPLC grade water solution was made by the method proposed by Liu and Hansen (1990). The detection was not made by the methods applied consequently no reason was found by the direct quantitative analyses.

There are many research activities about chitosan and usage of spectrophotometry and HPLC which are the applied methods to quantify. The lacking of the methods may be caused due to the not degrading chitosan to glucosamine monomer by hydrolysis since glucosamine can be determined by chromatographic, colorimetric and fluorimetric techniques (Eikenes et al., 2005).

4.5 Dynamic Vapour Sorption (DVS)

The experimental moisture sorption data obtained corresponding to the water activity values of the potassium sorbate, nisin and chitosan incorporated polymer films which ranges from 0 to 0.90 are presented in Figures 4.5, 4.6 and 4.7, respectively. The moisture uptake of the chitosan films had an increasing trend with increasing water activity (Figure 4.7) while the equilibrium moisture content of the potassium sorbate and nisin films were almost constant upto water activities 0.5 and 0.7, respectively. There were sharp rises after these water activities (Figures 4.5 and 4.6).

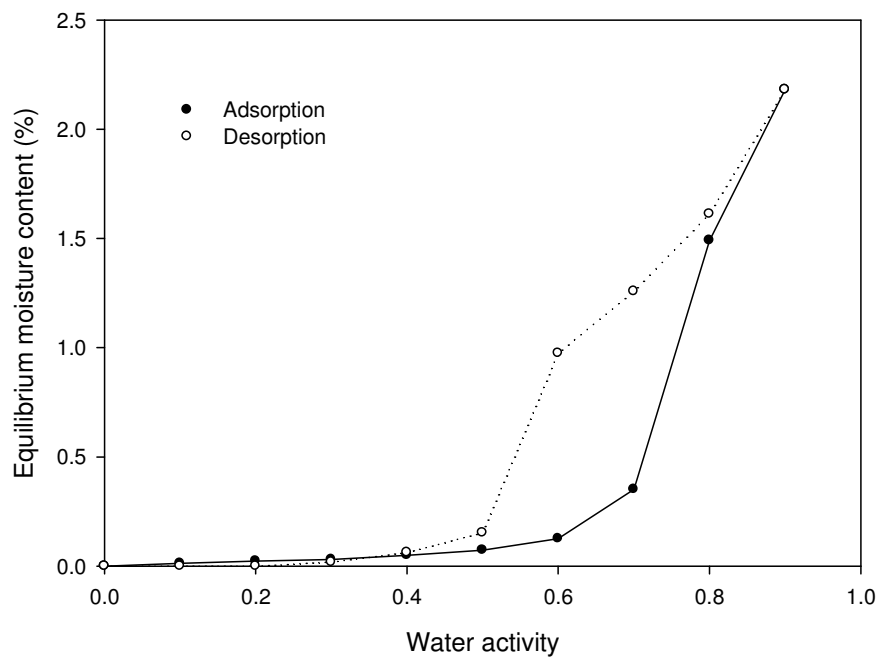


Figure 4.5 Moisture sorption isotherms of potassium sorbate 2% film.

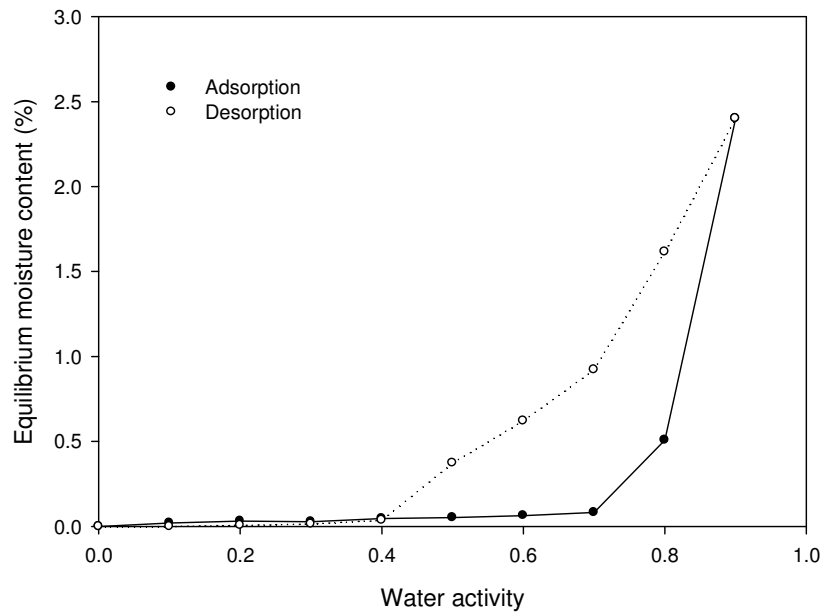


Figure 4.6 Moisture sorption isotherms of nisin 3% film.

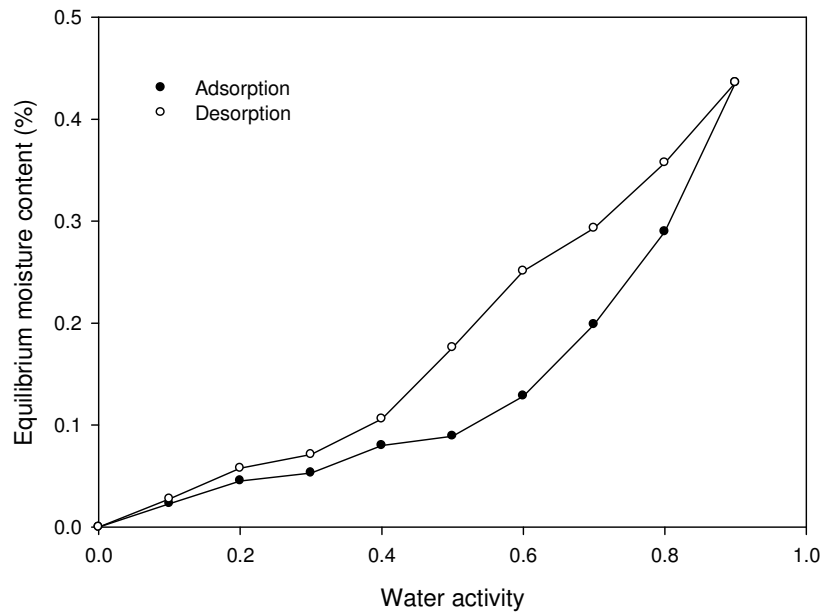


Figure 4.7 Moisture sorption isotherms of chitosan 3% film.

The equilibrium moisture content of the desorption plots were found to be higher than that of the adsorption, that caused hysteresis which was related to the nature and state of the food components, reflecting their potential for structural and conformational rearrangements and changes the energetically favourable polar sites accessibility. The presence of capillaries in food cause to significant decrease in

water activity. The explanation for the occurrence of moisture sorption hysteresis contains the molecular shrinkage theory (Raji and Ojediran, 2011). Hysteresis was observed in all films' sorption plots and was in a higher rate among the nisin, potassium sorbate and chitosan containing polymer films, respectively. Comparison of adsorption and desorption plots of potassium sorbate, nisin and chitosan incorporated polymer films were presented in Figures 4.8 and 4.9.

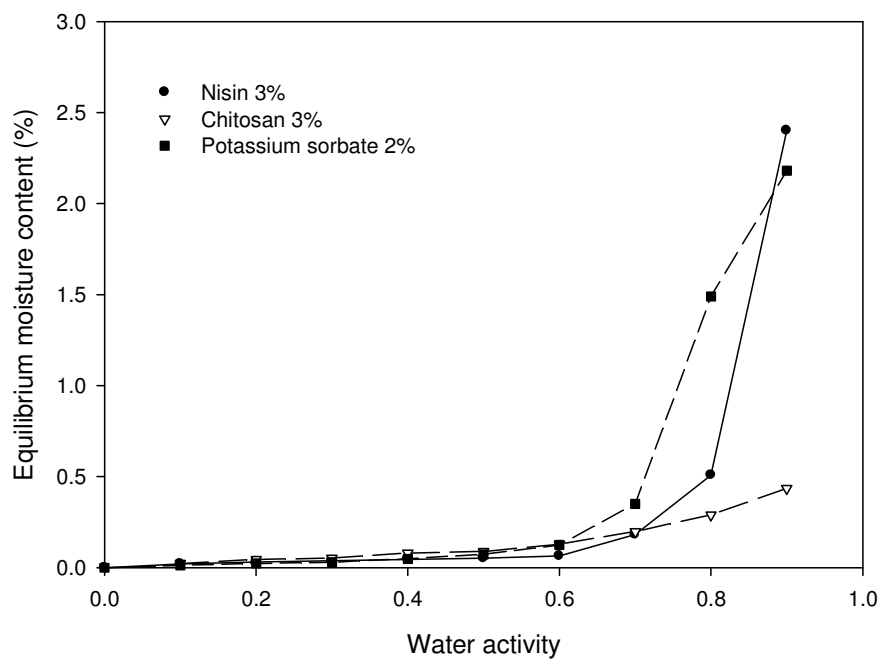


Figure 4.8 Comparison of adsorption of potassium sorbate, nisin and chitosan incorporated polymer films.

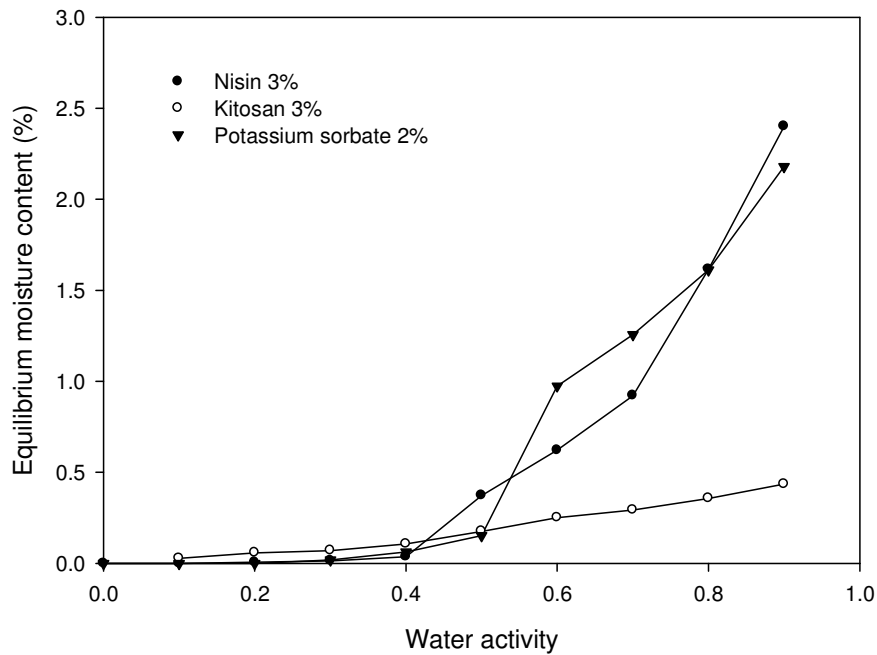


Figure 4.9 Comparison of desorption of potassium sorbate, nisin and chitosan incorporated polymer films.

The GAB model was used to describe the equilibrium moisture content and water activity relationship as it can be observed by the Figure 4.10, 4.11 and 4.12. The GAB parameters and the coefficient of determination R^2 were given in Table 4.3. It was determined that GAB model application was sufficient to fit the experimental data. The most important parameter in the model is the monolayer moisture content (m_0) explaining the minimum amount of water that could be absorbed in a single layer per gram of dry film (Strauss et al., 1991). It is represented at Table 4.3 that the monolayer moisture content of the polymer films containing 2% potassium sorbate, 3% nisin and 3% chitosan were 1.89, 0.29 and 0.09 at 20°C, respectively. It was found that potassium sorbate has the highest moisture uptake in spite of the lowest percentage of potassium sorbate. The reason of high water holding capacity may be attributing to that potassium sorbate is potassium salt of sorbic acid. On the other hand, nisin is a hydrophobic protein and has lower moisture uptake than potassium sorbate. The parameter K thermodynamically needs to be smaller than 1.0. The value of K provides a measure of the interactions between the molecules in the multilayer

with the adsorbent, and it tends to decrease between the energy value of the molecules in the monolayer and that of liquid water. In the study, it was represented at Table 4.3 that potassium sorbate has K value higher than 1.0.

Table 4.3 GAB parameters and coefficients of determination for dynamic vapour sorption of potassium sorbate, nisin and chitosan incorporated polymer films.

Film Types	GAB parameters				
	m_0	C	K	R^2	SSE
Potassium sorbate 2%	1.89	0.08	0.89	0.94	0.09
Nisin 3%	0.29	0.07	1.04	0.99	1.65×10^{-4}
Chitosan 3%	0.09	1.69	0.89	0.99	5.27×10^{-7}

m_0 : monolayer moisture content, C and K: adsorption constants, SSE: Sum of Squared Errors

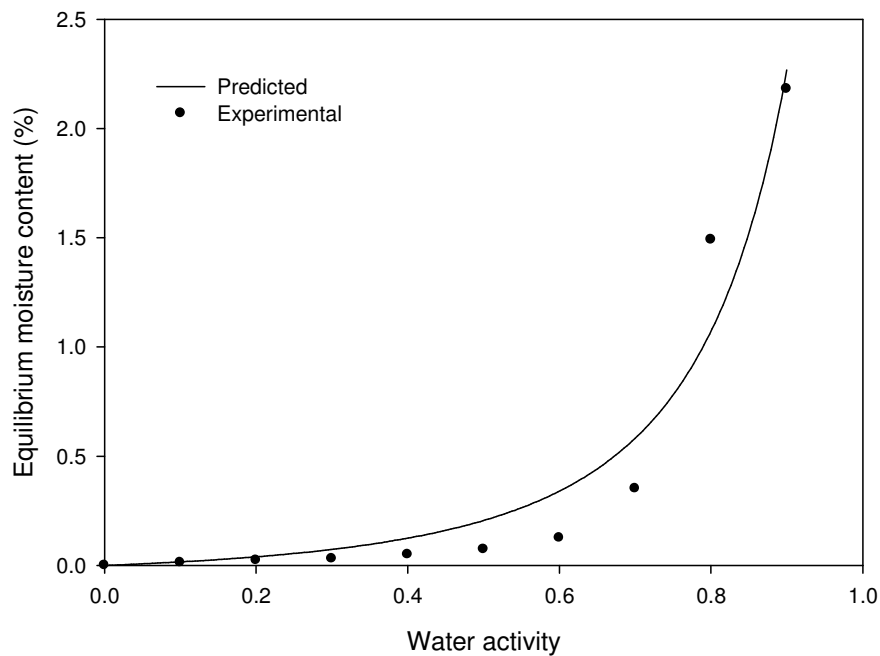


Figure 4.10 Application of GAB equation (Equation 2.9) to data of potassium sorbate 2% film.

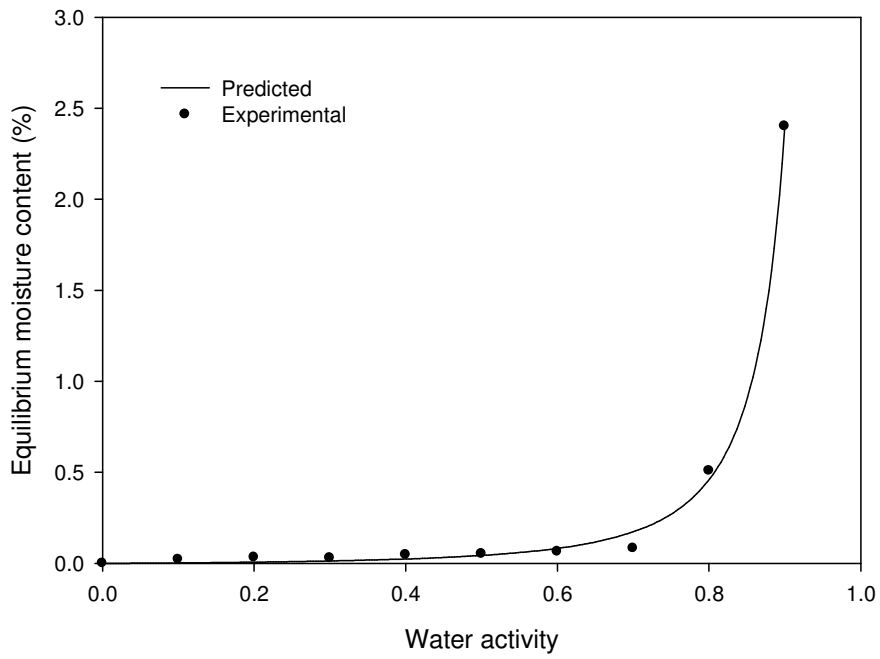


Figure 4.11 Application of GAB equation (Equation 2.9) to data of nisin 3% film.

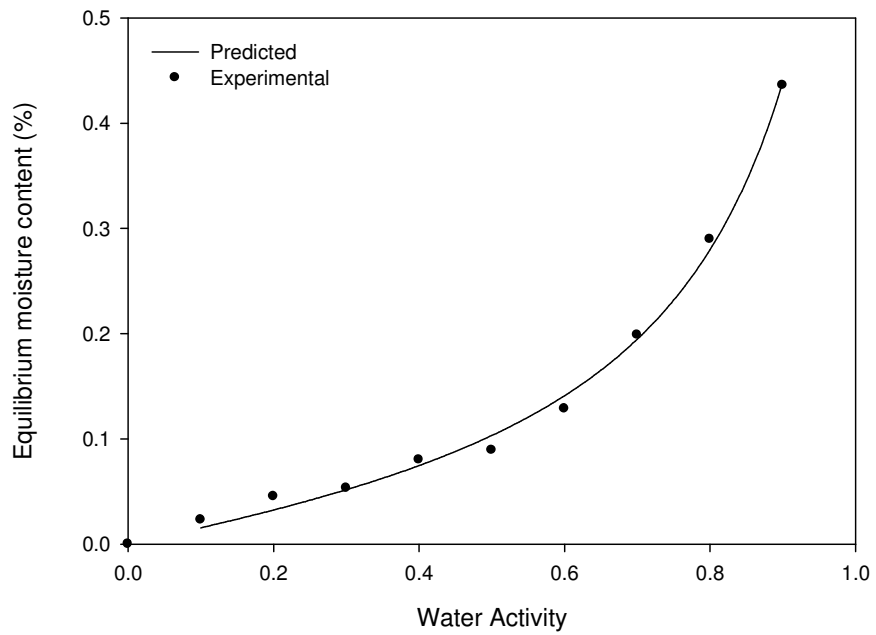


Figure 4.12 Application of GAB equation (Equation 2.9) to data of chitosan 3% film.

The DVS data was used to evaluate the amount of diffused water (M_t) at time t . The determined amounts divided with (M_∞) values. In this study (M_∞) values assumed as the amount of diffused water at thermodynamic equilibrium. (M_t/M_∞) versus $t^{1/2}$ was plotted to determine the k constant. Figures 4.13, 4.14 and 4.15 show that plot of (M_t/M_∞) against $t^{1/2}$ were linear for the potassium sorbate, nisin and chitosan containing packaging films. The slope of the linear regression gave k constant that would be used for determining the apparent diffusivity constants. Equation (2.8) was used to calculate D values. Table 4.4 shows that D values were found as 4.12×10^{-20} , 8.35×10^{-19} and 3.23×10^{-21} cm^2/s at 20°C , respectively for potassium sorbate (2%), nisin (3%) and chitosan (3%) between 70% RH and 80% RH. Since total moisture uptake was so lower in all film types at even at water activity 0.9, and the sensible moisture content was detectable after 0.7 water activities (Figures 4.10, 4.11 and 4.12). Hence it was decided that in order to compare the diffusivity of moisture through the active films with a high precision the equilibrium moisture content values after these range were used. The coefficients of determination were summarized also in Table 4.4.

It was found that the diffusion of moisture was higher in nisin containing polymer film than potassium sorbate and chitosan containing polymer films. Arce et al. (2003) was studied sorption kinetics of water vapor at poly (acrylic acid) (PAA) by using a gravimetric method. They measured the apparent moisture diffusivity constant as 3.5×10^{-8} cm^2/s at 69% water activity. They found high diffusion rate of water into polymer matrix because of PAA has hydrophilic nature. When water sorption kinetics of PAA and LDPE compared it was observed that water diffusion rate into PAA was greater due to nature of the polymer. Roy et al. (1999) was reported that the apparent moisture diffusivity constant of wheat gluten film as 1.1×10^{-4} m^2/day between 75 to 84% RH. As expected the moisture diffusion was higher through the hydrophilic wheat gluten film than low density polyethylene film.

In the study, potassium sorbate (1%), nisin (1%) and chitosan (1%) containing polymer films were also tested on DVS. The moisture diffusion in the polymer films was insufficient and the data obtained was unreasonable thus the apparent moisture diffusivity constants of potassium sorbate, nisin and chitosan (1%) incorporated films could not be calculated.

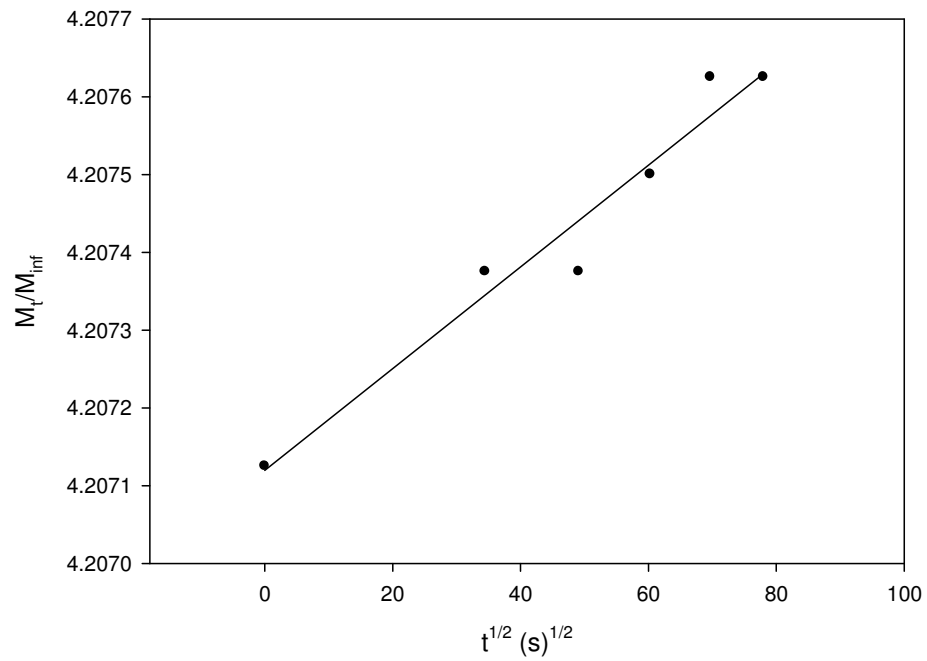


Figure 4.13 Diffusion plot of water in the range of relative humidity of 70% RH to 80% RH through potassium sorbate 2% films.

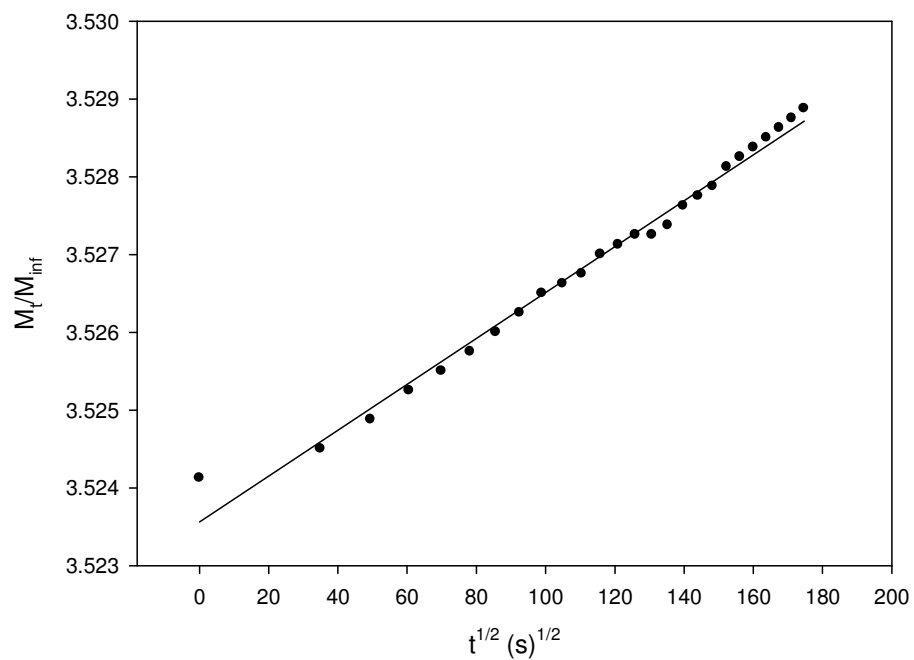


Figure 4.14 Diffusion plot of water in the range of relative humidity of 70% RH to 80% RH through nisin 3% films.

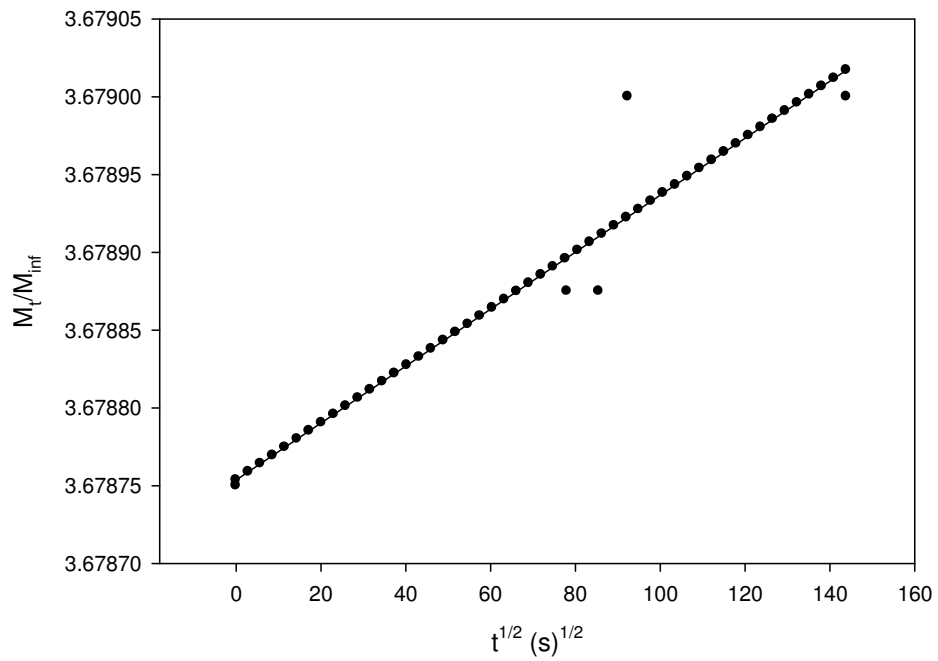


Figure 4.15 Diffusion plot of water in the range of relative humidity of 70% RH to 80% RH through chitosan 3% films.

Table 4.4 The diffusivity constants and coefficient of determination of potassium sorbate, nisin and chitosan

Film Type	D (cm ² /s)	R^2
Potassium Sorbate 2%	4.12×10^{-20}	0.95
Nisin 3%	8.35×10^{-19}	0.98
Chitosan 3%	3.23×10^{-21}	0.97

CHAPTER V

CONCLUSION

The study of properties and application of antimicrobial agent incorporated polymer films revealed the following conclusions:

1. The apparent diffusivity constant of potassium sorbate (1, 3, and 5%) and nisin (1, 2, and 3%) containing polymer films was found as 4.21×10^{-21} , 1.75×10^{-20} and 2.73×10^{-20} cm²/s at 25°C; 1.55×10^{-19} , 2.69×10^{-19} and 2.82×10^{-19} cm²/s at 35°C, respectively.
2. It is possible to say that nisin released in a higher rate than potassium sorbate through polymer films.
3. The GAB equation was used to fit the adsorption isotherms of polymer films and the monolayer moisture content (m_0) of polymer films containing 2% potassium sorbate, 3% nisin and 3% chitosan were 1.89, 0.29 and 0.09 at 20°C, respectively.
4. The apparent moisture diffusivity constants of potassium sorbate (2%), nisin (3%) and chitosan (3%) containing polymer films were 4.12×10^{-20} , 8.35×10^{-19} and 3.23×10^{-21} cm²/s at 20°C, respectively between 70% RH and 80% RH.
5. It was found that the diffusion of moisture was higher in nisin containing polymer film than potassium sorbate and chitosan containing polymer films.

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