RADIATION GRAFTING OF FUCTIONAL POLYMERS

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ABSTRACT

Non-migratory active food packaging has the potential to reduce the current food crisis which involves production, quality and safety aspects for consumption. Currently, the food industry is using added preservatives which possibly contain harmful ingredients that will enable food to last longer. Besides that, active packaging like cans or plastic contains wordings on the surface that can migrate into food product over a period of time hence it can be detrimental for the health of consumer. This work aims to prepare a film surface that will be used as packaging with non-migratory antimicrobial properties via gamma radiation grafting. Using a hydrophilic, biocompatible polymer polyethylene glycol diacrylate (PEGDA) as tether molecule. In this study, PEGDA was grafted onto the surface of polypropylene (PP) films which were irradiated using gamma ray with dosages ranging from 10kGy, 15kGy, 20kGy, 25kGy and 30kGy. The concentration for polymer solution is between 1% to 20%. In this study, N,N-dimethylaminoethyl methacrylate (DMAEMA) was used as an antimicrobial agent. It was later immobilized on the PP film surface which hve been grafted with PEGDA by covalent attachment. Grafting was done by dipping the irradiated films in the polymeric solution for two hours in water bath at 70^{o}C while purging with nitrogen. The weight of the films before and after grafting were taken and calculated using degree of grafting. After that, the films were characterized by using attenuated total reflectance Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and tensile testing machine. The result showed that a high degree of grafting of more than 10% was achieved when the total radiation dose was lower than 50kGy and the concentration for monomer solution of PEGDA and DMAEMA is kept below 20% within optimum condition.

ABSTRAK

Kaedah bungkusan makanan yang mampu mengelakkan penyebaran bakteria dari persekitaran mempunyai potensi untuk mengurangkan krisis makanan semasa yang melibatkan aspek pengeluaran, kualiti dan keselamatan. Ketika ini, industri makanan menggunakan bahan pengawet yang mengandungi bahan-bahan berbahaya untuk membolehkan makanan bertahan lebih lama. Selain itu, bungkusan yang diperbuat daripada tin atau plastik mengandungi label di permukaan yang akan menembusi ke dalam produk makanan dalam suatu tempoh masa dan perkara ini boleh menjejaskan kesihatan. Penyelidikan ini bertujuan untuk menyediakan bungkusan makanan yang mampu membunuh bacteria kerana sifat anti-mikrob yang tidak menembusi permukaan makanan. Proses ini dilakukan melalui radiasi gamma transplan. Dengan menggunakan sifat polimer yang hidrofilik dan ciri-ciri biologi yang serasi seperti polyethylene glycol diacrylate (PEGDA) sebagai lapisan untuk mencantum molekul. Dalam kajian ini, PEGDA dicantum kepada permukaan filem-filem polipropilena (PP) yang telah diradiasi dengn sinar gamma menggunakan dos 10kGy, 15kGy, 20kGy, 25kGy dan 30kGy. Kepekatan polimer adalah antara 1% hingga 20%. Dalam kajian ini, N, N-dimethylaminoethyl methacrylate (DMAEMA) telah digunakan sebagai agen anti-mikrob. Ia kemudiannya bercantum pada permukaan filem PP yang telah dicamtum oleh PEGDA melalui ikatan kovalen dengan kaedah radiasi yang sama. Proses cantuman dilakukan dengan mencelup filem-filem yang telah dikenakan sinaran radiasi dalam larutan polimer selama dua jam ke dalam bekas air yang direndam pada suhu 70^{o}C sementara proses penyingkiran oksigen oleh nitrogen dijalankan. Berat filem-filem sebelum dan selepas cantuman diambil dan dianalisi menggunakan tahap cantuman atau ’degree of grafting’. Selepas itu, filem-filem dianalisis menggunakan Fourier pantulan mengubah spektroskopi inframerah (FTIR), mikroskopi pengimbasan elektron (SEM) dan ‘tensile test’. Hasilnya menunjukkan bahawa tahap cantuman yang optimum melebihi 10% telah dicapai apabila dos sinaran adalah lebih rendah daripada 50kGy dan kepeketan untuk monomer PEGDA dan DMAEMA ditetapkan di bawah 20%.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

kGy	Kilogray
DMAEMA	N,N-dimethylaminoethyl methacrylate
PEGDA	Polyethylene glycol diacrylate
PP	Polypropylene
DG	Degree of grafting

1 INTRODUCTION

1.1 Motivation and statement of problem

Food is becoming more expensive and harder to obtain as a result of escalating land pressure and increasing population densities throughout the world (Oxby, 1985). The food crisis has made more people succumb to malnutrition and hunger especially countries in the African region. The safety of the food consumed is of utmost importance to ensure the general wellbeing of consumers is protected. Recent food borne microbial outbreaks and consumer demand to minimize chemical additives in food are driving a search for innovative ways to inhibit microbial growth in foods while maintaining quality, freshness, and safety.

In order to reduce autoxidation which causes food rancidity or spoilage, manufacturers of food and beverages company will add chemicals such as antioxidants (tocopherols, BHA, BHT, etc), reducing agents (ascorbate, erythorbate or ascorbyl palmitate) or chelating agents (citrate, phosphates or EDTA) during processing or to the finished product in order to extend shelf life. While these additives have been generally considered as safe when the total content is within the specific regulation of the Food and Drug Administration (FDA), at higher levels however they can be toxic and can endanger the life of humans (Schilderman et al., (1995); Witschi and Morse (1983)). For example, aspartame is a type of artificial sweetener that can affect intelligence and short-term memory. Besides that, regular consumption of monosodium glutamate as flavour enhancer can cause depression, disorientation, eye damage, fatigue and obesity. While sodium nitrate/nitrite, a toxic ingredient found in processed meat which can make old, dead meat appear fresh and vibrant can wreak havoc with internal organs and cause cancer as they are highly carcinogenic (Bosch, 2013).

Besides that, extensive research has been done on direct radiation on food, fresh fruits and vegetables. Normally, ionizing energy such as electron beams or gamma rays is used to inhibit sprouting of tuber, bulb and root vegetables which can reduce the nutrient level.

Although this treatment is most effective when applied during dormancy, there are several limitations to it such as undesirable changes in the structure and decreased wound healing property. Plus, the ripening of fruits can also be inhibited at certain doses to ensure good quality product. However, serious detrimental effects of such treatment have been observed such as uneven ripening, excessive softening and mushy fruits (Matsuyama and Umeda, 1983; Thomas 1984a; b). For, freshly meat however, it is not convenient to transport large amount of product to the radiation facility due to cost and effectiveness.

Research into the area of antimicrobial food packaging materials has increased significantly during the past decade. Approximately half of the 40 Food Packaging Division's technical posters presented at the '2001 Institute of Food Technologists Annual Meeting' (23-27 June 2001, in New Orleans, Louisiana, USA) involved antimicrobial packaging. Due to recent outbreaks of contaminations associated with meat products, growing concern over the safety of intermediate moisture foods, active packaging has been greatly explored in recent years. This has caused the red meat industry to increase the application of packaging containing preservatives. As mentioned by Appendini and Hotchkiss (2002), antimicrobial packaging is a technology that inhibits or retards the proliferation of microorganisms in foods, thus extending the shelf life of the product.

Principal active packaging systems involve oxygen scavenging, moisture absorption, carbon dioxide or ethanol generation, and finally antimicrobial systems. Indeed, ready-to-eat products such as cooked ham, are completely processed before final packaging and are consumed without further cooking, thus enhancing the possibility of the occurrence of food-borne illnesses if further contamination by pathogens occurs (Marcos, Aymerich, Monfort, and Garriga, 2007a). Moreover, as discussed by Skandamis and Nychas (2002), despite the extended shelf life of refrigerated products stored under vacuum pack or modified atmosphere packaging (MAP) conditions, there is an increasing concern about the growth and survival of microaerophilic and/or psychotrophic pathogens. Indeed, psychrophiles can grow within a temperature range of 14 ^{o}C-21 ^{o}C, with a high rate at 0 ^{o}C and an optimum growth at 15 ^{o}C or less. Meat and meat products may also be

contaminated by Listeria monocytogenes, Salmonella typhimurium, Salmonella enteritidis, Escherichia coli.

Basically, in order to lower the growth and rate of spoilage as well as harmful microorganisms in meat or other perishable foodstuffs, antimicrobial packaging materials could be improved by incorporating a non-migratory antimicrobial into the packaging because they are able to slow or even halt the growth of pathogenic microorganisms. Besides, this novel method will greatly enhance the safety of packaged food products and extend the shelf life of products (Han, 2005). Current application includes packaging films that release organic acids to reducing the effect of the growth of slime-forming bacteria on fresh meat (Rooney and Han, 2005). Films that are able to release lactic acid are very interesting because this acid exists in the meat and can be effective when used at the cut surface.

Although there are several advantages when combining biocide agents directly into a packaging material, it also has its downside due to migration of active substances that could have effects when consumed in large amount. Normally a high concentration is needed before the microorganism can be killed. Hence, this aspect is very dangerous for the safety of consumers as the accumulation can have side effects for the wellbeing of consumers. Besides, the direct incorporation of antimicrobial properties into meat or other perishable food products may result in partial inactivation of the active substances by the food constituents and is expected to have only a limited effect on the surface flora. On top of that the migration of antimicrobial properties into foodstuff is detrimental.

Figure 1-1 shows how a film with an antimicrobial coating could release the antimicrobial agent onto the surface of the food on the inside of the package. According to Cooksey (2001), the rate of release would depend on the type of interaction between the antimicrobial agent, the coating material, the targeted bacteria and the foodstuff itself. A barrier material might be necessary on the outside layer of the film to prevent loss of the antimicrobial additive to the outside of the package.

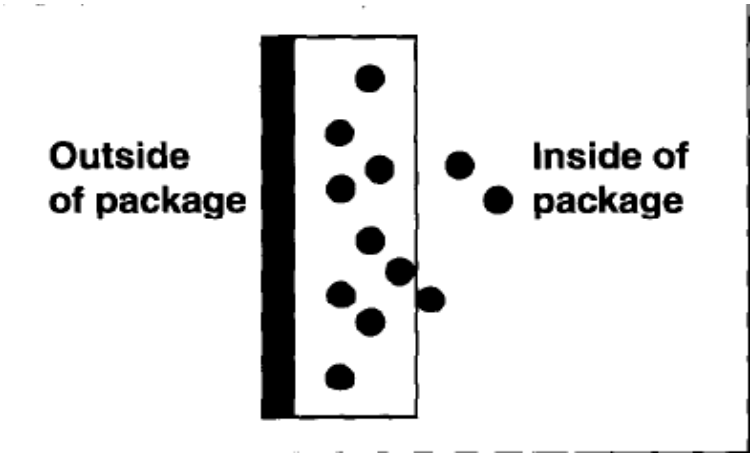

Figure 1-1: Antimicrobial agent could be released from a film which has the antimicrobial agent directly incorporated into the film.
Source: Cooksey (2001)

Hence, it is safe to say that manipulation of properties in a packaging material is of utmost importance in ensuring the quality and safety of food products. One of the most effective ways to achieve this is by incorporating a bioactive non-migratory antimicrobial compound onto the surface of active packaging systems. This will ensure that the bioactive compound will not be deposited onto the food product, and the safety of consumers will not be jeopardized as the activity will be sustained over a longer period. (Quintavalla and Vicini 2002; Ozdemir and Floros 2004; Goddard and Hotchkiss 2007).

In order to prevent migration and improve stability, the bioactive compounds are immobilized onto functionalized surfaces by covalent linkage using tether molecule which is not only hydrophilic but flexible as well (Endo and others 1987; Haynie and others 1995). In this research, the type of biocompatible tether polymer chosen polyethylene glycol diacrylate (PEGDA) while N,N-dimethylaminoethyl methacrylate (DMAEMA) was used as an antimicrobial agent that will be ingrained on the surface of films. This innovative technique can be applied to different kinds of polymer substrates and bioactive agents for the production of nonmigratory active packaging materials.

1.2 Objectives

- This work aims to produce a film surface with non-migratory antimicrobial properties via gamma radiation grafting.

1.3 Research Scopes

The following are the scope of this research:

i.) To analyze the optimum conditions for sample preparation, concentration of monomers and dose for grafting processes

ii.) To characterize films using Fourier Transform Series (FTIR), Scanning Electron Microscope (SEM), tensile strength analysis and determination of degree of grafting (DG)

1.4 Main contribution of this work

The following are the contributions:

- Non-migratory antimicrobial food packaging
- Storage for medical devices
- Gene carriers/ Drug delivery to body cells

1.5 Organization of thesis

The structure of the reminder of the thesis is outlined as follow:

Chapter 2 describes about the parameters used produce antimicrobial films that will be used for food packaging. Firstly, the type of backbone polymers chosen to attach the antibacterial properties play an important role. Next the steps to produce radicals which is needed for the grafting of polymers for properties enhancement. Besides that the type of bonding between the polymers are also studied as well as the type of antibacterial and antifouling polymer chosen to produce a novel food packaging system.

Chapter 3 discusses the method used to graft PEGDA and DMAEMA onto the surface of PP films via gamma-induced radiation. Starting from the material preparation such as polymer backbone PP films, material cleansing by sonication, drying using vacuum oven, weighing the weight of films before grafting, first batch irradiation of films which involves grafting of PEGDA onto PP films, PEGDA polymeric solution preparation, degasification by nitrogen gas, washing of homopolymer or unreacted monomer by distilled water, and reweighing the final weight of films. Next batch of irradiation of films involved grafting DMAEMA onto the same first batch of films with the same dosages applied, DMAEMA solution preparation, degasification, washing and finally reweighing the final weight of films after having been grafted with PEGDA and DMAEMA.

Chapter 4 discusses the results obtained from (a)grafting degree analysis, (b) SEM analysis, (c) FTIR analysis, and (d)tensile strength analysis

Chapter 5 draws together a summary of the thesis and outlines the future work which might be derived from the model developed in this work.

2 LITERATURE REVIEW

2.1 Overview

Since it is vital to produce a packaging that is safe for consumers, several parameters have been studied to determine the best way to produce antimicrobial films that will be used as active food packaging. Firstly, the type of polymers chosen as the backbone to attach the antibacterial properties play an important role. Next the steps to produce radicals which is needed for the grafting of polymers for properties enhancement. Besides that the type of bonding between the polymers are also studied as well as the type of antibacterial and antifouling polymer chosen to produce a novel food packaging system.

The effect of bacterial transfer into food products will not be covered in this research due to time constraint. Nevertheless, an optimum degree of grafting will be determined to produce non-migratory antimicrobial food packaging .

2.2 Type of Polymer

Packaging plays important roles for the safety of food such as preventing spoilage and contamination, extending the shelf life and ensuring safe storage right until consumption. Plastics have emerged as the most preferred choice for foodstuff packaging because it is the most cost effective medium compared to other materials. It is also easy to handle during transportations as the weight is lower and will be less damaging. On top of all, it offers better shelf life hence preventing wastage. Polypropylene (PP) was chosen because the properties can be changed to increase their strength, flexibility and improve the moisture or gas barrier (Allahvaisi, 2012).

According to Cooksey (2001), there are three basic categories of antimicrobial films:

(1) Incorporation of antimicrobial substances into a sachet connected to the package from which the volatile bioactive substance is released during further storage. Hence, common packaging materials can be utilized without the use of alternative packaging materials.

(2) Direct incorporation of the antimicrobial agent into the packaging film.

(3) Coating of the packaging with a matrix that acts as a carrier for the antimicrobial agent. These categories of materials can release the antimicrobial agents onto the surface of the food (Appendini and Hotchkiss, 2002; Buonocore, Del Nobile, Panizza, A., Corbo, & Nicolais, 2003; Halek & Garg, 1989; Sebti, Pichavant & Coma, 2002; Wen, Chen & Chen, 1999; Weng & Hotchkiss, 1992). The antimicrobial agents may either be released through evaporation in the headspace (volatile substances) or migrate into the food (non-volatile additives) through diffusion (Figure 2-1). The system is more efficient than a direct application of the antimicrobial agent onto meat surfaces, because it slows the migration of the agents away from the surface, and thus helps to maintain high concentrations where they are needed. Moreover, antimicrobial packaging can take two additional forms (Appendini & Hotchkiss, 2002):

(4) Utilization of inherently antimicrobial polymers exhibiting film-forming properties, such as cationic amino-polysaccharides (Begin & Van Calsteren, 1999; Coma et al., 2002; Ouattara, Simard, Piette, Be´gin & Holley, 2000; Pen & Jiang, 2003), or polymers which are chemically modified to produce bioactive properties. Packaging

materials with bioactive agents chemically bounded to the polymer can be included in this category and the immobilization of the biocide on the packaging polymer could be used for non-food-grade molecules such as medical equipments. However, the limitation of such system is the direct contact between the packaging and the food.

(5) Utilization of bioactive edible coatings directly applied onto the foods. The limitation is that the bioactive agent should be approved as a food additive. The antimicrobial packaging materials producing an atmosphere modification by adding a sachet into the package, potential of these technologies are evaluated to extend the shelf-life and assure the innocuousness and preservation of meat and meat products surveys the other antimicrobial packaging systems based on the dispersion or on the coating of bioactive agents in/on the packaging. It also surveys the utilization of inherently antimicrobial polymers or edible matrices. The potential of these technologies are evaluated to extend the shelf-life and assure the innocuousness and preservation of meat and meat products.

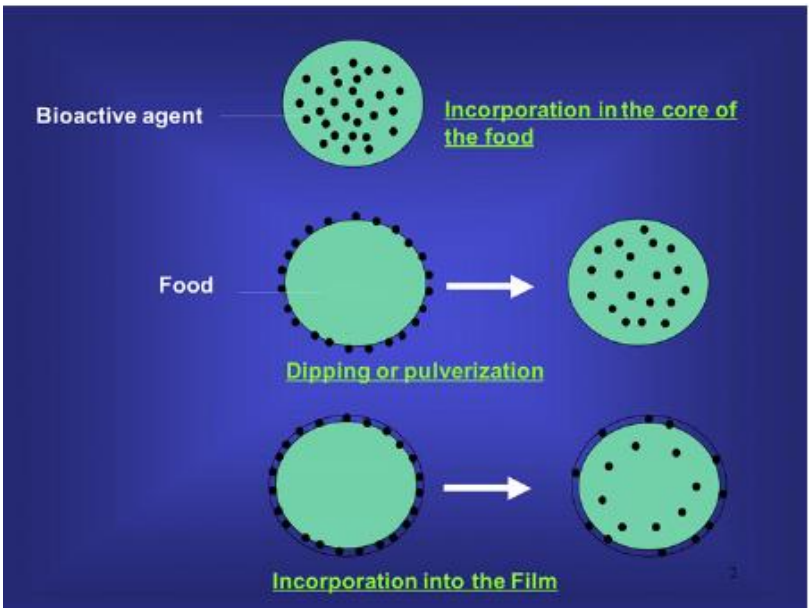

Figure 2-1: Different incorporation modes of additives in food products (incorporation into the foodstuff, dipping or pulverization, and finally incorporation into a film) and consequences. The black points correspond to an antimicrobial compound.

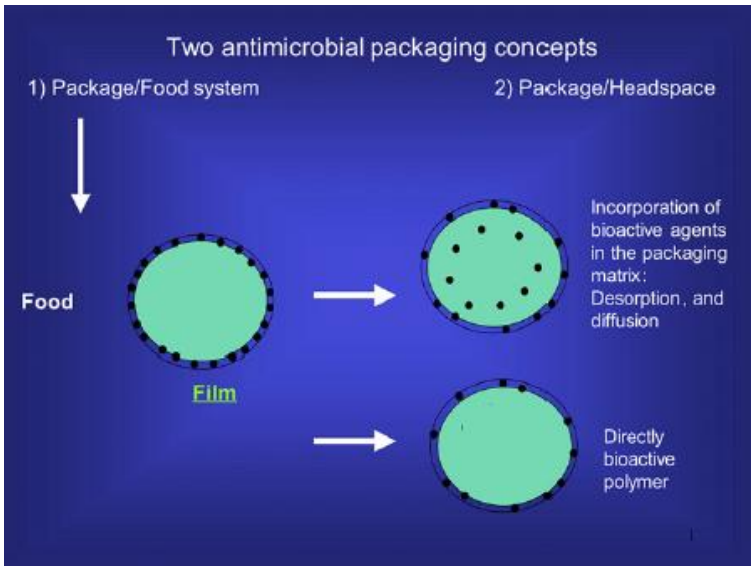

Figure 2-2: Antimicrobial packaging concepts: package-food and package-headspace systems

2.3 Radical Formation

There are several techniques that can be used to graft or crosslink polymers of different functional groups. It is either by chemical reaction or radiation. Examples of chemical routes to produce free radicals as initiators is by redox reaction using different reagent such as Fenton's reagent, persulfate and reducing agent, metal chelates, direct Oxidation and Chemical pretreatment (e.g., ozonation, diazotization, xanthation) of the polymer backbone. Another chemical method used is by using living radical formation whereby the chain termination step is avoided hence the polymer chain grow at a more constant rate. This can be achieved through atom transfer, nitroxide mediated, and degenerative transfer. In this research, crosslinking is achieved via high energy radiation such as gamma ray, X - ray, proton, electron beam, and neutron beams. The advantage of irradiation over chemicals is that it generates high concentration of free radicals, clean process with no waste, low energy consumption and basically easy to control (Bhattacharya, 2009)

2.4 Covalent Bonding Between Polymers

Grafting is achieved by covalently bonding polymers onto the backbone to impart various functional groups in order to improve its properties. Non-covalent adsorption of polymers to surfaces is a reversible process and such polymer brushes are often unstable. According to Bhattacharya and Misra, pre-irradiation free radical grafting means polymer backbone is irradiated in the presence of an inert gas or vacuum to form free radicals. In this method, monomer is not irradiated, so the homopolymerization is easier to be avoided. Next, the irradiated polymer(P) substrate is treated with the monomer (M), in liquid or vapor state or as a solution in a suitable solvent. The figure for pre-irradiation grafting is as follows.

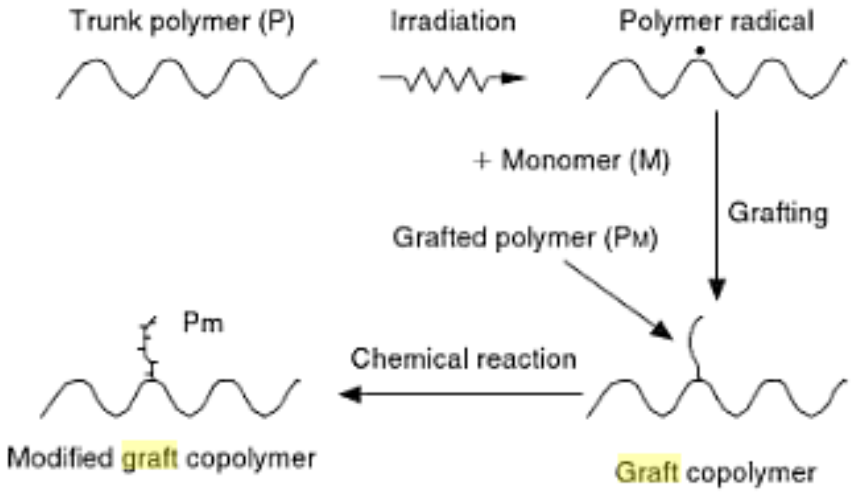

Figure 2-3: Irradiation grafting of functional polymers

2.5 PEGDA as Tether Molecule

Polyethylene glycol diacrylate (PEGDA) is chosen as tether to act as functionalized surface for covalent attachment of bioactive compounds such as antimicrobial agents, enzymes, and nucleic acids. This is because of its biocompatible, flexible and hydrophilic properties which can improve stability of the backbone polymer and allow for enhanced molecular mobility while avoiding regulatory implications and issues related to migratory systems in food (Mabrouk 1997; Manta and others 2003; Wang and Hsieh 2004). According to Lutz et al., another special nature of PEGDA is the antifouling surface which is normally used in biomedical devices, biosensors, microarrays, cell assay, tissue engineering, magnetic resonance image (MRI), filtration membrane, and microcapillary electrophoresis.

PEGDA is widely used in tissue engineering constructs made with synthetic polymers which allow users to control the mechanical and structural properties of the device. For example engineering of tissues including bone (Yang, et al. 2005), cartilage (Gabler, et al. 2009) (Hwang 2007), and cornea (Kadakia, Keskar, et al. 2008) (Myung, et al. 2008). Typical PEG hydrogel studies use a singular PEG chain size to adjust the structure's mechanical properties. (Lin, et al. 2011) (Myung, et al. 2007) (Sannino, et al. 2006). Hence, surface modification of a packaging material by grafting a flexible, hydrophilic tether molecule is therefore a versatile technique that can be applied to a variety of polymer substrates and bioactive agents for the design of nonmigratory active packaging materials (Endo and others 1987; Haynie and others 1995; Goddard and Hotchkiss 2007). From figure 2-4, it can be seen free radicals are produced from oxidation instead of via ionizing radiation. Next the polymer backbone (PE) is grafted with PEGDA and covalently bonded with amine-terminated molecule diamine via Michael's addition.

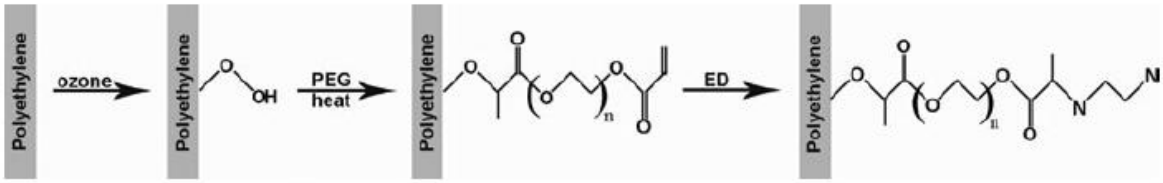

Figure 2-4: Experimental steps. Clean low density polyethylene (LDPE) films are exposed to ozone gas, followed by free radical graft polymerization of PEG diacrylate monomer. The amine-terminated molecule, ethylenediamine, is covalently linked to remaining acrylate groups via Michael's addition

Source: Goddard & Barish (2011)

2.6 DMAEMA as Anti-Microbial Polymer

N,N-Dimethylaminoethyl Methacrylate (DMAEMA) is a vinyl monomers containing tertiary amino groups chosen which is chosen as the polymer with antimicrobial properties. According to Aquino, they are also hydrophilic and can be polymerized or copolymerized via classical free-radical polymerization or by simple coupling reactions. This reaction is followed by quaternization, to induce the antimicrobial activity. Feng et al (2000) mentioned that DMAEMA is an active monomer which can be polymerized and copolymerized by simple coupling reactions or graft processes (free radical polymerization). Polymeric quaternary ammonium salt (PQAS) is one of the most commonly used antibacterial polymer types because of its excellent antibacterial activity, low toxicity and irritation, high penetrating effect and good environmental stability. This property has been considered to be a promising antimicrobial agent (Rawlinson et al.) According to Ignatova et al (2006), PQAS with a short alkyl chain from dimethylaminoethyl methacrylate(DMAEMA) or functional monomers poly(2-(*tert*-butylamino)ethyl methacrylate) PTBAEMA, and 3-sulfopropylmethacrylate (SPMA) has a stronger antibacterial ability compared to its precursory monomers. Feng, Brash and Zhu (2006) mentioned in their research that these surfaces are normally used in tissue engineering, contaminated medical devices, industrial and private settings, filtration, fibers, and microspheres. In this research, a novel way to utilize this special characteristic of DMAEMA is by applying its special characteristic in the food industry to package food products via gamma radiation method.

2.7 Summary

Basically research on non-migratory antimicrobial food packaging films are still lacking and the applications are very scarce. Hence this study is hoped to overcome the food crisis problem and produce a surface film that can be later modified to a safe packaging to kill or halt the growth of bacteria.

3 MATERIALS AND METHODS

3.1 Overview

This chapter discusses the method used to graft PEGDA and DMAEMA onto the surface of PP films via gamma-induced radiation. Starting from the material preparation such as polymer backbone PP films, material cleansing by sonication, drying using vacuum oven, weighing the weight of films before grafting, first batch irradiation of films which involves grafting of PEGDA onto PP films, PEGDA polymeric solution preparation, degasification by nitrogen gas, washing of homopolymer or unreacted monomer by distilled water, and reweighing the final weight of films. Next batch of irradiation of films involved grafting DMAEMA onto the same first batch of films with the same dosages applied, DMAEMA solution preparation, degasification, washing and finally reweighing the final weight of films after having been grafted with PEGDA and DMAEMA.

3.2 Chemicals and raw material

All the raw materials used in this research were available at Malaysian Nuclear Agency, the Department of Radiation Processing of Natural Polymer and Nanomaterials. The chemicals were obtained from Sigma Aldrich with very high purity (>90%). The facility for gamma irradiation was within the compound which uses Cobalt-60 as source of radioactive material. The chemicals used are poly(ethylene glycol) diacrylate (PEGDA), N,N-Dimethylaminoethyl Methacrylate (DMAEMA), ammonium iron(II) sulphate (Mohr's salt), propanol, acetone, ultra pure water and distilled water. While the backbone polymer polypropylene (PP) were obtained from the stationary shop in the shape of certificate folder.

1**.) Material Preparation**

- PP films were cut approximately 5x5cm. The surface and properties of these films were modified by grafting PEGDA onto the PP films and immobilizing DMAEMA on the surface.

Figure 3-1: PP films which have been cut to 5x5 cm

2.) **Material Cleansing**

- All the films were thoroughly cleaned in order to ensure good dispersion of free radicals on the surface and good covalent linking between the backbone PP polymer, PEGDA and DMAEMA.
- A sonicator was used to rid the films of any dirt before sending for irradiation.
- The films were placed inside a bottle containing 250ml of propanol and then placed in a sonicator for 30minutes.
- The propanol was poured out and replaced with acetone and sonicated for another 30minutes.
- Lastly, the propanol was replaced with the same amount of ultra pure water and sonicated again for 30minutes

Figure 3-2: Solution inside a sonicator

3.) **Drying**

- The films that have been cleaned with tissues were placed in a number of petri dishes.
- All the films were placed in a vacuum oven and heated for 70˚C for 2 hours.

Figure 3-3: Dry films in petri dishes