Studies on Metallocene Polyolefin and Polyvinyl Chloride for Blood and Blood Component Storage Applications

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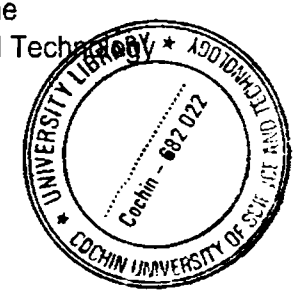

BY
M. C. Sunny

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CERTIFICATE

This is to certify that the thesis entitled **"Studies on Metallocene Polyolefin and Polyvinyl Chloride for Blood and Blood Component Storage Applications"** is an authentic report of the original work carried out by Mr. M. C. Sunny under my supervision and guidance in the Department of Polymer Science and Rubber Technology, Cochin University of Science and Technology, Cochin-682 002. No part of the work reported in this thesis has been presented for any other degree of any other institution.

Prof. Dr. K. E. George
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11-07-06

DECLARATION

I here by declare that the thesis entitled ***"Studies on Metallocene Polyolefin and Polyvinyl Chloride for Blood and Blood Component Storage Applications"*** *is the bonafide report of the original work carried out by me under the guidance of Prof. Dr. K .E. George, Department of Polymer Science and Rubber Technology, Cochin University of Science and Technology, Cochin 682 022, and no part of this thesis has been presented for any other degree of any other institution.*

Cochin
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M. C. Sunny

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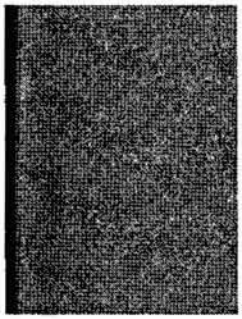

PREFACE

Plasticized poly(vinyl chloride) (pPVC), although a major player in the medical field, is at present facing a lot of criticism due to some of its limitations like the leaching out of the toxic plasticizer, diethylhexyl phthalate (DEHP) to the medium and the emission of an environmental pollutant, dioxin gas, at the time of the post use disposal of pPVC products by incineration. Due to these reasons, efforts are on to reduce the use of pPVC considerably in the medical field and and to find viable alternative materials.

The present study has been undertaken in this context to find a suitable material for the manufacture of medical aids in place of pPVC. The main focus of this study has been to find a non-DEHP material as plasticizer for pPVC and other suitable materials for the complete replacement of pPVC for blood/blood component storage applications. Two approaches have been undertaken for this purpose – (1) the controversial plasticizer, DEHP has been partially replaced by polymeric plasticizers (2) an alternative material, namely, metallocene polyolefin (mPO) has been used and suitably modified to match the properties of flexible PVC used for blood / blood component storage applications. The thesis is presented in seven chapters.

Chapter 1: The first chapter begins with a literature survey on the use of flexible PVC in medical field. The present position of PVC in medical field, limitations of PVC, advantages as well as limitations of plasticized PVC in medical products etc are discussed. Literature survey also covers the various methods adopted to overcome the limitations and the possibility of replacing flexible PVC with suitable alternative materials

Chapter 2: The details of the various materials and experimental techniques employed in this study are described in this chapter.

Chapter 3: The plasticizer DEHP has been partially replaced with three polymeric plasticizers namely Acrylonitrile Butadiene Rubber(NBR), Epoxidised Natural Rubbber(ENR) and Carboxylated Nitrile Rubber (XNBR). Various blend compositions have been prepared and properties of each of the blends have been compared with those of control (DEHP plasticized PVC). Effects

of temperature on plasticizer leaching and permeability properties were carried out with PVC/-NBR, the most promising system. Cytotoxicity evaluation of this blend has also been undertaken.

Chapter 4: The suitability of m-PO for blood/ blood component storage application is assessed in this chapter by comparing its properties with those of plasticized PVC and ethylene vinyl acetate copolymer (EVA), the two materials presently used for blood or blood component storage applications.

Chapter 5: In order to improve the gas permeability, mPO has been blended with highly gas permeable EVA. Two grades of EVA with different vinyl acetate contents (12 and 18wt %) were blended with mPO. Mechanical properties like tensile strength, percentage elongation at break, and tear strength, percentage volume swelling, clarity of the blends were evaluated with respect to EVA content. Amounts of extractables and the pH of the medium were also analysed. The gas permeability and water permeability of these blends have also been determined.

Chapter 6: The most suitable candidate for blood and blood components storage, mPO modified with EVA12 (75:25) was further analysed for the biological performance. Biological evaluation of the blends is carried out as per the procedures detailed in the International Organization for Standardization (ISO)-10993. In vitro cell culture cytotoxicity studies (direct as well as indirect test methods) on mouse fibroblast cell line (L929) were carried out as a preliminary screening test. Haemolysis, cell adhesion studies and blood-clotting time were then performed using blood from human volunteers.

Chapter 7: The summary and conclusion of the whole study are presented in the last chapter.

Contents

Chapter 1 INTRODUCTION

1.1 Role of PVC in medical sector

4

1.2 Flexible PVC medical products

7

1.3 Limitations of Flexible PVC

1.3.1 Toxic effluents during manufacturing.

1.3.2 Plasticizer Leaching

1.3.3 Dioxin and Hydrochloric acid emission during the disposal of PVC by incineration.

1.3.4 Chemical interaction with drugs or package contents.

9

1.4 Human Exposure to DEHP

11

1.5 Toxicity of DEHP and its metabolite

1.5.1 Effect of DEHP Exposure on Younger Childrens

1.5.2 DEHP and Biocompatibility

INTRODUCTION

Introduction

Polymers are the most important and largest family of materials used in medical technology (1-5). With the advent of plastics in the field of medical technology, the use of traditional materials such as glass and metals has been reduced considerably. The utilization of plastics in the medical disposable device sector minimized or avoided the risk of cross-contamination and infection and the need for resterilization (1.6). Various polymers like Poly(vinyl chloride) (PVC), Polyethylene, Polypropylene, Polystyrene, Polycarbonate, Acetal copolymers, Poly(butylene terephthalate), Poly(ethylene terephthalate) and some specialised polymers synthesised through biotechnology route are used for different medical applications. Of these, poly(vinyl chloride) is the most widely and extensively used plastic for disposable medical products (1,7,8). Its use has grown considerably over the past 60 years, to the point where PVC represents more than 27% of all plastics used for the manufacture of medical devices (9,10). The breakdown of various plastics used for different applications in medical sector is shown in Figure 1.1.

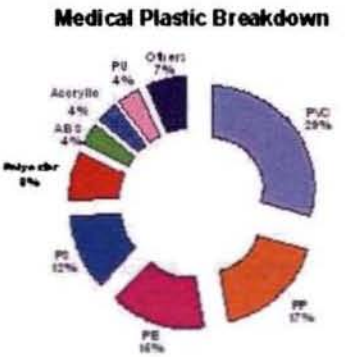

Figure 1.1 Breakdown of plastics used in medical sector.

1.1 Role of PVC in medical sector

Poly(vinyl chloride) is the leading polymeric material used in medical devices and packaging applications in terms of total volume consumed. Its rise to dominance in the medical market is part of a larger trend in the displacement of glass, aluminum, and metals by plastics. Light weight, shatter-resistant, and easy-to-handle, plastics have pushed aside the traditional materials for holding, storing, transferring, and dispensing biological liquids. Among the various plastics materials, PVC has dominated alternative polymers because of its optical clarity, flexibility, surface finish, capacity to withstand steam sterilization (up to 121°C — also referred to as autoclaving), ability to form tight seals through radio frequency (RF) sealing, resistance to kinking, biocompatibility with a range of solutions and body tissues, and especially low cost. These excellent and varied properties made PVC one of the most prominent materials for medical device manufacturing.(7,11-15). In most of these applications, PVC has captured a share of 70 to 90% of the total market.

PVC is a relatively rigid and brittle amorphous polymer (7). In the amorphous structure, the individual molecules of the polymer lack mobility because of the strong chemical bond between hydrogen and chlorine atoms of adjacent polymer chains. Flexible PVC plays a major role in medical device manufacturing. The specific characteristics of vinyl medical devices are achieved through the addition of a wide variety of additives. Around 98% of the total consumption of PVC for medical applications is used for the production of flexible products like bags, tubes, gloves etc (Figure1.2).

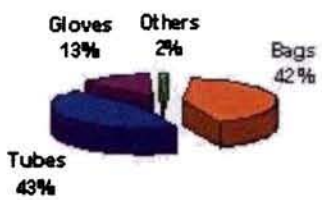

Figure 1.2 Breakdown of flexible PVC products in medical sector

Flexibility of PVC can be achieved by blending with plasticizers. Plasticizers are generally clear, organic, liquid materials that are added to PVC formulations to obtain flexible products. The use of a plasticizer involves the introduction of a lower molecular weight substance into the structure that acts as a molecular lubricant, physically separating the chains and allowing them some mobility, thus giving the flexibility (16,17). In order to get the property of flexibility the plasticizers should be highly compatible with polymer resin and should become an integral part of the matrix. Plasticizers allow PVC to be softened and shaped into many designs without cracking (16,18). Obviously, larger volume of plasticiser increases the flexibility and softness of the material (12). In reality, the mechanism of plasticisation is a little more complex. The incorporation of a plasticiser into PVC involves the penetration of the plasticiser into PVC resin particles, which causes them to swell. During this process, the polar groups in the PVC are separated and polar groups in the plasticizer are able to interact with those of the resin. The structure of the resin is then re-established with full incorporation of the plasticiser in the polymer structure. This effectively provides 'free volume' in the polymer that allows for the molecular flexibility. Unplasticised PVC has negligible free volume. The incorporation of plasticizer decreases the interaction forces of adjacent chains, lower the glass transition temperature of the polymer and produce chain mobility and material flexibility. It is important to note that the nature of the plasticiser molecule, in relation to its molecular size, polarity, solid-gel transition temperature and the precise characteristics of the plasticiser – polymer interaction, controls the effectiveness of the plasticiser, both with respect to the flexibility introduced and to the retention of the plasticiser in the material. Many putative plasticisers fail to interact with the PVC resin and produce little or no flexibility, whilst some give flexibility but the final structure is such that the plasticiser cannot be retained under operational conditions and is lost over time, causing a reversion to the brittle state.

There are several different types of plasticisers that can be used in PVC (19). These include adipate esters, phosphate esters, citrates, trimellitate esters, sebacate and azelate esters and

phthalate esters. These vary in their characteristics and performances, each having relative advantages and disadvantages under different circumstances. Chemical formula and molecular weight of PVC and some common plasticizers are given in table1.1.

Table 1. 1 Chemical Formula and Molecular Weight of PVC and common plasticizers.

Chemical Name	*Formula*	*Molecular Weight*
PVC	$H\text{-}(CH_2CHCl)_n\text{-}H$	100,000
DOA	$C_{22}H_{42}O_4$	370
DEHP	$C_{24}H_{38}O_4$	390
ATHC	$C_{26}H_{46}O_8$	486
BTHC	$C_{28}H_{50}O_8$	514
TEHTM	$C_{33}H_{54}O_6$	546

By far the greatest volume of plasticiser used in PVC is accounted for by the phthalate esters. It has been used in PVC formulations since 1930. About 95% of phthalates produced are used as plasticizer in PVC (20). It became the choice plasticizer for PVC because of its easy-to-process, easy-to-disperse, and low cost. It is the most widely used PVC plasticizer in the world. So DEHP is regarded as the international standard plasticizer for PVC (21). Di(2-ethylhexyl) phthalate (DEHP or DOP by another name) is the most commonly used plasticizer in flexible PVC products (22,23). DEHP is a colorless, odourless, and lipophilic oily liquid that is essentially insoluble in water (0.3 mg/l) (24). But it dissolves in most organic solvents and is miscible with many mineral oils and lipids such that it is reasonably soluble in body fluids (18).

DEHP has been found to be a highly compatible plasticizer for PVC resin (16,25). The PVC - DEHP system has extensively been explored for the production of many industrial, household as well as medical products (26-29).

Because of its ability to provide medical devices with their desired mechanical properties, DEHP became the common plasticizer in medical field also (7). It is the main plasticizer approved by the US Food and Drug Administration for medical uses (30). The European Pharmacopoeia also recommended DEHP as a softening agent for disposable medical items such as blood bags and tubing (31). The structure of DEHP is given in the Figure1.3.

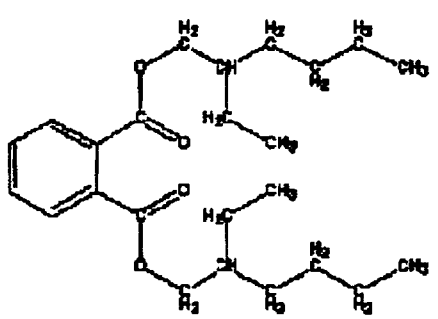

Figure. 1.3. Structure of DEHP

The following sections provide a brief overview of the specific examples of flexible PVC products utilized in many clinical settings. Its uses are numerous and extremely varied as shown below.

1.2 Flexible PVC Medical Products

Flexible Bags

Infusion bags, dialysis bags, blood bags, secretion bags and urine bags are come under this category. Infusion bags are used for the storage of drug solutions; sugar solutions and electrolyte solutions, while the function of blood bags and transfusion bags are for blood withdrawal, treatment, storage and transfusion. Multipurpose bags are used for preparing erythrocyte concentrate and for preparations in closed systems, and also for the process known as plasmapheresis, in which the erythrocytes are transfused back into the donor after the plasma has been extracted. There are two forms of urine bags: disposable types and complicated multirouting systems, which are sometimes connected to measurement equipment, for example in hourly urine, flow measurement systems. Dialysis bags contain sterile electrolyte solutions, which are introduced via catheters into the abdominal region of the patient and are retained there for several hours before being exchanged.

Tubings

A wide variety of PVC tubing systems is used for infusions of drug solutions, sugar solutions, electrolyte solutions etc. and transfusions of blood and blood components, and also for irrigation and evacuation procedures via catheters.

Feeding Equipment

Nasogastric tubes are used in large numbers for short term feeding of neonates. Many other components are also included in this category, involving bags and tubes for the delivery of liquid food products.

Respiratory Support Equipment

A wide variety of components is used to assist patients in respiration, including oxygen masks and tubes, endotracheal and tracheostomy tubes, nasal cannulas, humidifier equipment, and resuscitator and ventilator components.

Organ Assistance

Some major medical procedures aimed at short, medium or long-term functional assistance to organs involve PVC tubes and components. This includes extracorporeal membrane oxygenation (ECMO) and haemodialysis.

Catheters

Many short-term catheters and drains are used in the clinic, including umbilical vessel catheters, wound drainage tubes and osteotomy shunts.

Products with Skin Contact

Examination gloves are obviously used very extensively in clinical and laboratory procedures and they employ a wide variety of materials, including plasticised PVC. A wide selection of other products, including identification bracelets are made of PVC and come into direct contact with the skin of patients.

Products with Oral Mucosal Contact

Orthodontic retainers are used in young children, typically between 7 and 14 years of age, in order to prevent misalignment. An 'active' retainer may be used over several months for several hours during the day and at night. Retainers may also be used to stabilise the teeth in a predetermined position as the final part of orthodontic treatment, in which case they may be used for longer periods of time per day, possibly over several years. The use of these retainers is widespread. Orthodontic retainers may be prefabricated and made for phthalate plasticised PVC.

General Hospital Equipment

A number of fixtures of the hospital and clinic environment are constructed from

PVC, including wall and floor coverings and screens.

The high compatibility of DEHP with PVC has been shown in medical products like sheets and tubing where more than 50% of DEHP is used for the required flexibility (32). A study of DiGangi found that some medical devices contain between 29% and 81% DEHP by weight as indicated in Table1.3 (33). But DEHP has no chemical attachment to the PVC chain; it is only dispersed in the polymer matrix and bound to the plastic only by van der Waals forces (32).

Table 1.2 DEHP in Vinyl Medical Products

Item	*Head*
Blood Bag; mfr: Baxter	65
Catheter; mfr: Baxter	41
IV Bag; mfr: Baxter	42
IV Bag; mfr: Abbott	39
Syringe in IV Tubing; mfr: Abbott	29
Syringe in IV Tubing; mfr: Baxter	30
Tubing; 36" Arterial pressure tubing mfr: Abbott	29
Tubing in Blood Bag; mfr: Baxter	67
Tubing in IV Bag; mfr: Abbott	81
Tubing in IV Bag; mfr: Baxter	35

[mfr, manufacturer;. Determinations by State Analysis, Chicago, IL. Results shown are the averages of duplicates. The average coefficient of variation was 9.8%. Source: DiGangi]

1.3 Limitations of Flexible PVC

Despite all the superb attributes and wide applications of PVC, it has been at the centre of a controversial debate during the last two decades. A number of diverging scientific, technical and economic opinions have been expressed on the question of PVC and its effects on human health and the environment. Over the past several years, a number of publications concerning toxic emissions, effects of plasticizers on animals and humans have been reported. Most commonly cited shortcomings of PVC are:

1. Toxic effluents during manufacturing.
2. Leachable plasticizer
3. Dioxin and HCl generation upon incineration.
4. Chemical interaction with drugs or package contents.

1.3.1 Toxic effluents during manufacturing.

Poly(vinyl chloride) contains more than 50 percent chlorine by weight. As a result of PVC's relatively high chlorine content, its production, use and disposal give rise to emissions of dioxin, vinyl chloride monomer, and other dangerous, chlorinated organic pollutants. Vinyl chloride, the essential building block of PVC is a proven human carcinogen. Workers in PVC production facilities and residents of neighboring communities are inevitably exposed to vinyl chloride emissions.

1.3.2 Plasticizer Leaching

Plasticizer leaching is a serious problem in flexible PVC products. As the plasticizer is not chemically bound to the polymer it is leached out from the products during storage or while in use. The loss of plasticizer due to the leaching is found to affect adversely the function of the product in two ways i). The mechanical properties such as abrasive, compressive and tensile strength of the polymer article changes considerably ii). the flexibility and the transparency of the product loose drastically. Leaching of DEHP from any given PVC product is dependent on many factors which include: concentration of the phthalate in the PVC matrix (greater concentration leads to more leaching), vapor pressure of the plasticizer (greater vapor pressure leads to more leaching), surrounding temperature (greater temperature leads to more leaching), size of the sub-chains, presence of secondary plasticizers to reduce leaching, the degree of "curing" of the plasticizer and polymer, the type of surrounding media, application of pressure or agitation, and storage or use time (10,33,34).

In modern medical technology many plasticized PVC devices come in contact with blood, blood components or medical drug solutions while undergoing different medical procedures such as hemodialysis, transfusion of whole blood, platelets or plasma, extracorporeal oxygenation, cardiopulmonary bypass, and administration of intravenous fluids (35-39). During these medical procedures there are chances of leaching out of DEHP into the surrounding media such as blood or blood components, IV fluids or medications (40-42). This extraction occurs either by the

DEHP directly leaching out of the PVC product or when an extracting material (blood, IV fluids) diffuses into the PVC matrix, dissolves the plasticizer and the two diffuse out together (43-46). The overall loss of plasticiser from PVC products will depend on both the diffusion constants with respect to the PVC and the solubility or volatility at the surface (47).

Certain drugs are found to accelerate the DEHP leaching from PVC intravenous (IV) bags into solutions (48-53). In the Handbook on Injectable Drugs, Trissel (1998) identified a wide range of drugs that have been shown to increase the leaching of DEHP from IV bags as mentioned in Table 1.3 (52) Trissel noted that the presence of surface-active agents or large amounts of organic co-solvents in the formulation might enhance leaching of the plasticizer. The nutrient fluids used in these bags have a high lipid content, which would likely increase the leaching of DEHP (54).

Table 1.3 Drugs that increase the leaching of DEHP from PVC bags into solution

Name of the drug	*Application*
Etoposide, Paclitaxel, Teniposide	Chemotherapeutic
Chlordiazepoxide HCL	Antianxiety
Micronazole	Antifungal
Cyclosporine, Tacrolimus	Immunosuppressive
Fat Emulsions and Vitamin A	Nutritional

Source: "Hand Book of Injectable Drugs", Trissel (1998)

1.3.3 Dioxin and Hydrochloric acid emission during the disposal of PVC by incineration.

As the numbers of disposable medical products in use increase, the public concern about the environmental and biological risks of their disposal will also grow.

Dioxin, the most potent carcinogen ever encountered, is created during all phases of PVC production, as well as in its disposal by incineration or accidental fire (55,56). Dioxin is made up of 75 different compounds. A more complete name for these compounds is chlorinated dibenzo-p-dioxins (CDDs), which are commonly referred to as polychlorinated dioxins. Municipal waste incinerators are a leading source of dioxin, and half of the chlorine in incinerators that ends up in dioxin comes from PVC waste.

Dioxins are extremely toxic and potent environmental contaminants. They modulate and disrupt multiple growth factors, hormones, immune system and developmental processes (57-60). Over the last few years, an increasing number of reports have appeared about the reproductive problems in both wildlife and humans. Nanogram to microgram/kg body weight doses of dioxin on a single day during pregnancy was found to cause permanent disruption of male sexual development in rodents, including delayed testicular descent, lower sperm counts, and feminized sexual behavior (61). The study of The American Public Health Association revealed that a prenatal exposure to dioxin in rodents substantially increased the risk of breast cancer later in life (62).

1.3.4 Chemical interaction with drugs or package contents.

The DEHP that has been leached out from IV bags may interact with the content of the solutions stored in, which in turn affect the delivery of appropriate amount of therapeutic drugs and thus alter their effects on human. These interactions may have important implications in the patients. Petersen, et al. (1975) found that DEHP might compete for the same binding protein sites as the drug dicumarol, significantly increasing the coagulation time of mouse blood (63). They further demonstrated that DEHP increases hexabarbital (a barbituate) sleep time in rats by increasing the retention time for hexabarbital (due to DEHP's lipophilic characteristics). Mahomed, et al. (1998) found that the concentration of the drug diazepam (Valium) fell to around 80% in glass and to 50% in a PVC container after four hours and to an even lower concentration in the PVC container after eight hours of storage (64).

1.4 Human Exposure to DEHP

Humans are exposed to DEHP through multiple sources and routes: ingestion, inhalation and intravenously (65,66). As a result of the continuous release during the production, use and disposal of PVC products, phthalates are often described as the "most abundant man-made environmental pollutants" (67). DEHP migrates at a constant rate from plastics to the environment. It is detected in water, soil and food and therefore it is considered as a widespread environmental contaminant. Due to environmental contamination, bioaccumulation along the food chain and leaching from packaging during its storage, food is contaminated with DEHP. But the extent of contamination is more in the case of fat containing food like fish, milk, oils etc. (9,68). Even drinking water is being contaminated with DEHP while carrying through PVC pipes. Human can be exposed to DEHP by oral administration through the ingestion of DEHP contaminated food and water. In the case of small children, ingestion of DEHP may also occur through chewing or sucking on PVC products especially toys and theeters (69-71).

In medical field the DEHP exposure is mainly through medical devices. According to Huber, et al. and other researchers, human exposure to DEHP through medical treatments can be substantial. Administration via inhalation is another source of DEHP exposure and is mainly through respiratory support equipments. A wide variety of PVC components like oxygen masks, and tracheostomy tubes, nasal cannulas, humidifier equipments and resuscitator and ventilator components are being used to assist patients in respiration (72,73). Latini and Avery (1999) documented a loss of 0.06-0.12 mg DEHP per mg of endotracheal tube sample (6%-12%) after use (74). They further imndicated the degradatkion of material and its loss of flexibility after a few hours of use. Hill et al also measured DEHP in gases passed through DEHP-containing PVC tubing used for respiratory therapy (75).The main source of DEHP exposure during different medical procedures may be from the flexible products like bags and tubes as it contribute the lion share of flexible PVC medical products.(76-79). The quantitative assessment of DEHP leaching was further done by many research workers (17,79). Jaeger and Rubin in 1970 first quantitatively assessed the concentration of DEHP leached out (36). They found that 6 mg of DEHP per 100 ml of blood and its anticoagulant solution after being stored in PVC blood bags for 21 days at 4°C. DEHP migration found to be decreased significantly at –20°C. The

leaching of DEHP into whole blood was again confirmed by recent reports and the authors further detected a small quantity of mono ethyl hexyl phthalate (MEHP), the metabolite of DEHP (80-82).They further notice the leaching of DEHP and its conversion to MEPH was high in platelet-rich plasma (DEHP up to 181 mg / l and MEHP up to 31 mg / l) and even more in platelet concentrates (180-650 mg / l and up to 76 mg / l, respectively). According to their findings the leaching of DEHP and its conversion to MEPH was in the order of Whole Blood < Platelet rich plasma < Platelet concentrates. Since DEHP is converted to mono (2-ethylhexyl) phthalate (MEHP) by plasma esterases (83-84), blood and blood products contaminated with DEHP may also contain MEHP. Although there is very little data on human metabolism, it is generally believed that human metabolism of DEHP is similar to that of primates (52).

Leaching of DEHP from PVC tubing, used in a large number of medical applications such as haemodialysis, extracorporeal membrane oxygenation, cardiopulmonary bypass procedures, IV delivery of TPN emulsions, enteral feeding, and mechanical ventilation of infants and adults was quantitatively assessed and reported (44,85-87). The absorption, distribution, metabolism and excretion of DEHP have been thoroughly reviewed by Huber et al.(10)) and, more recently, by the NTP-CERHR Expert Panel Report (88).

1.5 Toxicity of DEHP and its metabolites

This section reviews the adverse effects of DEHP and its metabolite, monoethyl hexyl phthalate (MEHP), on several organ systems including the reproductive system(89-91), the kidney (92-95), the heart (96-97), the lungs (98-99), the liver (100-102), and hormonal changes (103) based on animal studies. Most of the initial information about the toxicity of DEHP comes from toxicological studies on animals. To determine whether an exposure to a chemical is harmful to humans, direct human evidence is the most useful, but this is very hard to come by because it is obviously not ethical to conduct experiments in humans, and, observation of humans exposed through daily life is difficult for a variety of reasons. Moreover, it is difficult to link delayed effects with earlier exposures. The results from animal testing at high doses are then extrapolated to human exposures at lower doses. In toxicology, the results obtained from animal testing, supplemented by in-vitro (out side the body) testing on human and animal cell lines, are generally considered relevant to humans, though the magnitude and types of effects may vary between species. When a substance has been shown to be toxic in laboratory animals, it is often later shown to be toxic in humans unless metabolic pathways in animals are very different.

Although the impacts of DEHP exposures have not been well studied in humans, DEHP has been for many years suspected to be potentially harmful to human health. This conclusion is based on animal studies that were believed relevant for predicting human impacts.

However, some in vitro tests showed abnormal functions of the blood components stored.

In 1988 Labow et al.(104) showed inhibition of human platelet phospholipase A2 and decreased platelet function in stored platelets. In 1993 Fredricsson et al. (105) observed that long-term exposure to DEHP resulted in a 25% reduction of human sperm mobility and Calo et al. (106) showed an increased secretion of interleukin-1 in DEHP-stimulated mononuclear human cells that could be, at least in part, responsible for peritoneal sclerosis in subjects undergoing peritoneal dialysis. In 1995 Smith et al. (107) observed acute toxicity in cultured human lung fibro-

blasts, probably subsequent to the synergistic action of plasticizer and stabilizer, while in 1998 Fischer et al. (108) showed a dose-dependent impairment in leukocyte oxidative metabolism in vitro. More recently, an increase in lipid peroxidation of erythrocytes and consequent hemolysis, presumably due to oxidative damage to the cell membrane, as well as a decrease in the concentration of vitamin E in blood stored in DEHP-plasticized bags have been reported (`109-111).

The Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency identified the reproductive toxicity of DEHP (112).

Colón et al. (2000) reported elevated levels of several phthalates [including diethyl phthalate (DEP), DBP, and DEHP] in serum samples from young girls with premature breast development (113).

1.5.1 Effect of DEHP Exposure on Infants

Studies on animals have shown that younger developing animals are more vulnerable to testicular toxicity than the older ones (114-117). It has been indicated that children would absorb chemicals more efficiently, process them more slowly and eliminate them less efficiently than adults (118). In another study, Parke showed that neonates were often more receptive than adults to the toxic effects of chemicals (119). Infants in neonatal intensive care units are regularly exposed to high doses of DEHP following various medical procedures. Because of their smaller size, children and especially premature and small infants may receive a larger dose of DEHP on a milligram per kilogram basis than adults when the same-size medical device is used for all ages. Therefore, potential exposure risk to DEHP will be presumably higher for infants, particularly infants at an early and more sensitive stage of their development

The toxicokinetics of DEHP are potentially quite different in very young and premature infants. In mature humans, like in rodents, DEHP is metabolized to MEHP by pancreatic lipase and absorbed through the gut. It is then glucuronidated and excreted, resulting in little or no tissue accumulation. In infants, pancreatic lipase systems are not fully mature until 6 to 12 months of age. (120-122). Neither premature nor full-term infants have mature glucuronidation until about 3 months of age (123). Thus, this important clearance mechanism for MEHP is not fully available to neonates and young infants, and may lead to slower MEHP excretion and higher levels of MEHP in neonates than in older children and adults (124). In infants, DEHP exposure mainly occurs in Neonate Intensive Care Units (NICU) during treatments. (53,125). Calafat et al conducted a study to determine whether premature infants who undergo various medical procedures were at increased risk of exposure to DEHP than the general population (126). This study provides the first quantitative evidence confirming that newborns who undergo intensive therapeutic medical interventions are exposed to higher concentrations of DEHP than the general population. Newborn infants could be exposed to more than 4 mg per kg of DEHP per day through blood transfusions (46).

FDA has been identified the following groups at greatest risk on DEHP exposure. The greatest risk groups are newborns including premature newborns, infants and young children, ECMO patients, cardiopulmonary bypass patients, exchange transfusion infant and children patients, patients receiving certain IV therapies, particularly those on TPN and those receiving lipophilic drug formulations. Additional groups possibly at risk include trauma patients receiving multiple

blood transfusions, haemodialysis patients, oxygen therapy patients, and children of breast-feeding females, pregnant women and pre-pubescent males.

1.5.2 DEHP and Biocompatibility

The ability of polymers to induce platelet aggregates, platelet adhesion and thrombus formation during in vivo blood-material contact is well known and the lack of these undesirable effects is considered very important in order to assess biocompatibility of biomaterials (127). It has been shown that DEHP can influence the biocompatibility of PVC medical devices and this might have detrimental effects on exposed subjects. In fact, since 1976 it has been known that DEHP might increase platelet adhesion and aggregation due to the greater adsorption of γ -globulin and fibrinogen (128).

Moreover, in 1995 Kicheva et al. (129) reported that an increased amount of DEHP causes material deterioration in PVC drain tubes, inducing the formation of thrombi on the surface of material in vitro as well as in vivo and decreasing blood coagulation time and hemoglobin concentration in exposed dogs. Likewise, in 1999 Zhao and Courtney (130) observed that a reduction in the amount of surface plasticizer improves the blood compatibility of plasticized PVC. In addition, More recently, Lamba et al.(131) reported the role of DEHP-plasticized PVC as a potent compliment activator – a process associated with adverse haematological effects – when it comes in contact with the human blood.

Many investigators have proposed to discontinue the use of PVC medical devices containing extractable materials, which could be even slightly harmful and to make every effort to find harmless additives, especially when they are used for children and pregnant women.

1.6 Earlier attempts to reduce the DEHP leaching

A strong controversy over the toxicity of DEHP persists all over the world. Since early 1980s, alarming reports questioning the safety of using DEHP as a pasticizer in medical PVC applications have been surfacing. Many investigators have addressed the issue of DEHP leaching and attempted to control/reduce the leaching of DEHP. Techniques such as grafting, coating and surface cross-linking with dithiocarbamates in order to reduce or inhibit plasticizer migrations are among the most attractive ones (132-134). In one of the earlier attempts, Miyamoto and Sasakawa showed that the leaching of DEHP could be inhibited by glow discharge treatment of PVC products (135). In another study, the reduction of DEHP migration was done by modifying the base polymer by radiation or chemical grafting using various monomers and standardized certain methods for reducing the amount of plasticizer migrating into blood and blood products (136). A different approach has been recently proposed by Jayakrishnan and Lakshmi, involving surface modification of DHEP-plasticized PVC articles by reacting with sodium sulfide in the presence of a phase-transfer catalyst (137). This treatment hinders DHEP migration and leakage in the surrounding medium. However, tedious processing steps and high cost hindered the success of these attempts on an industrial level.

In another attempt the plasticizer DEHP tried to replace with many other plasticizers like adipates, citrates, trimillitate etc. In 1980 Jacobson et al tried Hatcol-200 as a plasticizer in

medical PVC. The rate of Hatcol-200 leaching from PVC by serum was found out by radioassay method and reported to be one hundredth of that of DEHP(138).Citrates, based on naturally-occurring compounds, are the most costly alternative and are more water-soluble than DEHP. One alternative that has been approved for use in blood bags is butyryl-n-trihexylcitrate, which is metabolized to butyric acid, hexanol, and citrate. Use of this material in other medical applications has not yet been approved, and before it can be used in applications such as ECMO circuits, studies to evaluate durability (ie, flexibility and strength of tubing in the circuit) over time are needed. Adipates are even more water-soluble, and though they provide greater low-temperature flexibility than DEHP, they are more likely to exude. Trimellitates are less water-soluble than DEHP, less volatile, less oil-extractable, and less likely to mar or craze styrenics and polycarbonate; but they do not have FDA 21 CFR listing.

A comparison of Plasticizers for Use in Flexible Vinyl Medical Products has been carried out by Adams in 2001 (139). And looks at the various mechanisms under which plasticizers can leave flexible PVC medical devices. It also reviews available information on the migration and extraction characteristics of various plasticizers, including DEHP, trioctyltrimellitate (TOTM), citrates, and adipates, and discusses potential selection criteria based on plasticizer permanence.

1.7 Recent Reports of Government and Other Organizations

There are a wide range of initiatives that have been taken to avoid or restrict the use of PVC over the years globally, including measures taken by local authorities, hospitals and companies (140). Government organizations and expert panels have reviewed the subject of DEHP toxicity in recent years and some have drawn specific conclusions and recommendations. These are summarised as follows.

The European Union

Various opinions of the Scientific Committees of the European Commission have dealt with DEHP and PVC in recent years, most notably concerning the Scientific Committee on Toxicity, Ecotoxicity and Environment (CSTEE) (141). The same committee has reviewed the risk assessment of the European Chemical Bureau (ECB 2001) and agrees with the majority of the conclusions of that assessment (CSTEE, 2002), both with respect to the environment and to human health (142).

The European Parliament Resolution

On 26 July 2000, the European Commission published a Green Paper on PVC The Green Paper assessed various environmental and health issues related in particular to PVC waste management and presents a number of options to reduce those impacts (143).

In 2001, the European Parliament in a resolution called on to protect human health and the environment from the hazards posed by PVC, and to introduce rapidly a policy on the replacement of soft PVC and the Parliament further suggested that the PVC industry should look into the possibility of setting targets for reducing the use of phthalates, particularly in medical equipment.

In 1997, European Union appointed Sweden and its Chemical Inspectorate (KEMI) to complete the risk assessment and risk reduction strategy. The risk assessment report was agreed and finalized in September 2001 after numerous consultations with a broad range of experts. The Center for the Evaluation of Reproductive Risks to Humans (CERHR) constituted an expert panel to analysis of the developmental and reproductive risks to humans of 7 phthalate esters, including DEHP under the direction of the US National Toxicology Program. The CERHR expert panel expressed minimal concern over the exposure to the general adult population. Whereas the panel expressed "concern" that infants and young toddlers, because of their dietary preferences and mouthing behaviors, might have higher exposures to DEHP at a time when the male reproductive tract is still developing and potentially vulnerable (144). The CERHR expert panel expressed "serious concern" that critically ill boys undergoing intense medical or surgical treatment might receive higher doses of DEHP and MEHP due to the multiple simultaneous medical exposures and that could damage the reproductive tract. Of similar concern was the possibility that pregnant and lactating women might deliver higher levels of DEHP and MEHP to their infants via placental transfer and breast milk than is estimated for the general population, which is potentially more dangerous to males with developing reproductive tracts (145).

The US Food and Drugs Administration

The US Food and Drug Administration recently issued reports that reiterate the concern that some subpopulations of medically exposed individuals, including highly exposed male infants, could be at risk of testicular toxicity from exposure to DEHP (146). The Public Health Notification by FDA's Department of health & human services by July 12, 2002 commented the use of DEHP plasticized PVC as follows (147)

The manufacturers should consider to eliminate the use of DEHP in such devices that can result in high exposure in sensitive patients and that certain products be labelled with their DEHP content. Advice would be given on how manufacturers should deal with PVC in relation to regulatory procedures. Further recommended to use PVC Devices that do not contain DEHP or to use the devices made of other materials such as ethylene vinyl acetate (EVAC), silicone, polyethylene or polyurethane. The new FDA document is a very important signal to medical device manufacturers that it's time to move away from PVC - which not only leaches toxic phthalates during use, but also creates the potent carcinogen dioxin when it is manufactured and burned - and onto the next generation of materials.

Health Canada

An expert advisory panel was formed to advise Health Canada on the scientific evidence for any risk in relation to DEHP in medical devices, and possible actions that would reduce or eliminate exposure, and therefore any possible risks (148). The panel reported in January 2002 (Health Canada, 2002). Their recommendations included the following:

- Alternative products such as heparin-coated tubes should be used for all extracorporeal membrane oxygenation (ECMO) procedures in neonates and infants,
- Tubing and storage bags used for the administration of lipophilic drugs that contain surfactants should not contain DEHP,
- Total parenteral nutrition (TPN) solutions should be administered to newborns and infants only via products that do not contain DEHP.
- Research into further methods for reducing the release of DEHP from medical products should be urgently encouraged.

1.8 Phase Out of PVC

Due to the growing public concern about its alleged toxicity, the use of PVC has been reduced considerably in medical field and the medical product manufacturers are trying to switch over to alternative materials (149). The research community is in search of a material, which would match all the good qualities of plasticized PVC. A significant new development in the IV bag market is Baxter International's pledge to phase-out PVC use in IV solution bags (150) that reflects the trend in the industry toward non-PVC materials. According to the Memorandum of Understanding signed on 5 March 1999 by Baxter which committed to exploring and developing alternatives to PVC products and is developing and implementing proposed timetables for substituting its current containers for intravenous solutions (IV) with a container that does not contain PVC. The Japanese company Terumo has begun manufacturing PVC-free dialyzing fluid bags made from polypropylene and has developed a new polypropylene material for continuous ambulatory peritoneal dialysis; McGraw Inc supplies PVC-free IV bags in the US; and Saint-Gobain Performance Plastics has recently developed an alternative to PVC for medical tubing (151).

The announcement by United Health Services that they will seek to replace PVC medical supplies with cost effective alternatives reflects this trend within the healthcare provider community. The increasing concerns about DEHP and PVC coupled with regulatory or government policy declarations and market trends away from PVC in certain flexible applications forced for an increased research and development into alternative polymers.

Many hospital allover the world took announced their initiative to decrease or cease the use of PVC (152-,153).

1.9 Availability of Alternative Materials

PVC has been under attack for its perceived environmental and health risks since 1973. But the search for a replacement material for flexible PVC started only in the year 1980. Vicent (1981) in his review about medical tubings narrated the pros and cones of many materials that would be a possible replacement material for PVC (2). Among the materials investigated were polymers such as polyethylene, polypropylene, polyurethane and other polyolefins, silicone, ethylene vinyl acetate, flexible polyesters, various thermoplastic elastomers, polyolefin blends Silicone-polycarbonate block coplymers and multi-layer laminate plastics (154). They are inherently flexible, thus do not require a softening agent. Since they do not contain phthalates or any other additives, there will not be any concern of DEHP exposure and the danger of becoming brittle after long-term use. In addition, using non-PVC medical alternatives avoids the life-cycle hazards associated with PVC.

Ethylene vinyl acetate copolymer

EVA copolymer is a soft, clear and transparent material that can be used for containers and tubing. It can be available in a wide range of hardness depending upon the vinyl acetate content. EVA can range from the thermoplastic (conventional plastic) to elastomeric (rubbery) state by modifying the content of the polymer. Polymer impact strength can also be adjusted by the adjustment of vinyl acetate. This means that EVA can be processed for a wide variety of applications without the use of additives. Plasticizer leaching and subsequent lowering of the material's performance are absent in EVA. Due to its polarity EVA sheets can be easily welded by radio frequency technique and its low sealing temperatures reduce the processing cost. High cost and tackiness compared to PVC are the main disadvantages of EVA.

Polyolefins

DEHP plasticized PVC will confront many market challenges in the near future in most medical device and other product markets from non-PVC polymers and non-DEHP plasticized PVC polymers. Non-PVC polymers, especially the polyolefins, showed its potential in plasma and platelet packaging applications and pushed PVC out of the IV bag market. "Polyolefins" are a class of polymers that include the polyethylenes and polypropylenes. They are currently the most widely used commodity plastics in the world because of their ease of processing, low cost and durability. It can be made into products of varying hardness by modifying the polymer, without the use of plasticizers. Although it contains anti-oxidants, different research groups indicated that no anti-oxidant traces were detected in the platelet concentrates after 5 days of storage at 22°C and shaking (155,75). B. Braun/McGaw already has almost 20 percent of the IV market and uses polyolefin-based, multi-layer bags. Baxter, the leading user of PVC bags with almost 50 percent of total PVC medical bag consumption, plans to phase-out PVC use in IV bags. In the blood bag market, polyolefins pose a serious threat to PVC in the plasma and platelet markets where they already have significant market share. Baxter uses polyolefin plastic containers for the storage of blood platelets and pre-mixed drugs for intravenous injection.

Polyethylene

Polyethylene notable for chemical inertness, comparative softness and good surface characteristics is available in low and high-density grades. The latter distinguished by greater hardness and stiffness. The high permeability of CO2 does not comment its use for storing whole blood or red blood cells. Polyolefin bags compete with the TETM plasticized PVC bags in the storage of platelets and platelet rich plasma. The polyolefin bag can also store platelets for five days and is cheaper than the TETM plasticized bag.

Polypropylene

Polypropylene is both harder and tougher than the polyethylenes and is noted for its extremely high flex resistance. Resistance to irradiation sterilization is poor and during storage after irradiation there is a considerable risk of oxidative degradation and embrittlement, which can be critical for thin-section profiles. U.S. Pat. No. 4,479,989 discloses that, although plastic formulations including polypropylene are heat-sterilizable, they are undesirable since they may not be radiation-sterilized (156). Furthermore, plastic formulations should remain flexible at low temperatures during storage. But the polypropylene homopolyers, or copolymers, or blends thereof, are disclosed as brittle at low temperatures and inherently stiff. .

Polyurethanes

Polyurethane (PU) is available in a range of hardness from rigid to IRH 80°. Initially clear with a light straw cast, on processing there is a rapid deterioration to a brown colour in uv light and on continued processing. Kinking resistance is poor even worse than EVA. It is autoclavable but a sharp softening transition makes thermoforming difficult. Heat welding is easily accomplished and bonding is reasonably straightforward with dioxan. In processing it tends to stick to itself leading to trouble in take off and reeling. High water vapour permeability of PU adversely affect it use as fluid storage containers (157). Moreover, the cost of the material is very high compared to PVC. Therefore thermoplastic polyurethane cannot be recommended as a replacement material.

Silicones

Medical grade silicone rubber is still the most biocompatible material in use. Available in wide range of hardness; the surface properties and biological inertness minimize the possibility of damage to blood cells and tissue. It is thermo set and cannot be softened by heat. Silicone rubber is not a material for choice for a huge volume application like medical bags because it cannot be welded, is very notch sensitive and moreover is very expensive.

A wide variety of medical uses of plasticized PVC are based upon a combination of many characteristics. All these characteristics can individually be replicated by the some or other above-mentioned materials, particularly with respect to the mechanical characteristics that give the flexibility and softness coupled with reasonable strength and toughness. But no effective PVC-free material is yet available for widespread blood product storage application.

Coextruded films

Coextruded polyolefin films have also been tried in recent years to replace PVC for medical infusion bags and blood storage containers. These films are typically based on coextrusions of polyolefin skins—such as polypropylene (PP) or linear-low-density polyethylene (LLDPE)—with HF-active core layers, such as polyester (PET) or nylon. Additional PP films comprising blends or coextrusions with styrene-ethylene/butylene- styrene (SEBS) or TPU were the other materials tried in place of PVC(158-161). Although these films exhibit good physical properties, barrier properties, HF weldability, and, in some cases, autoclaveability, they have apparently not yet found widespread commercial use because of their high cost.

Cost of the material is also an important factor while considering an alternative material to the cheap PVC products. In the report commissioned by Green peace International in 1999, Joel Tickner made a cost-analysis of the alternative materials to that of PVC(162). Several of the alternative materials listed in the report were found to be cost competitive with PVC. However, since the parts are usually made by volume and not weight, a simple comparison of the price per unit weight would be misleading. Figures of comparative volume cost are an important issue to consider.

Several efforts have been made to develop plastic material suitable for storing blood components from non-PVC plastics. Surprisingly, many of the materials tested, while giving indications of being good plastic materials for the manufacture of blood bags, showed high plasma hemoglobin content in the stored blood. This indicates that the lysis rate of the red blood cells in these containers is high. Examples of blood bags made from plastic formulations other than PVC are disclosed in U.S. Pat. Nos. 4,301,800; 4,479,989; and 5,026,347 (157,163-164).

While Baxter remains the leading user of PVC for bags and tubing, it also uses PVC-free materials in some applications. For example, Baxter uses polyolefin plastic containers for the storage of blood platelets and pre-mixed drugs for intravenous injection; uses EVA containers for nutritional lipid solutions and bone-marrow banking; and uses other PVC-free polymers for the storage of blood plasma and frozen blood cells (165,166). Baxter is also developing a polypropylene/ polyethylene blend for extreme temperature medical applications. Baxter uses EVA for the manufacture of nutritional lipid solutions containers. Corpak's Polar Enteral Feeding Bags are made from a polyethylene/nylon laminate and EVA. The bag has little market share due to higher cost and little or no value added in terms of increased shelf life. Shelf life is a critical factor driving material selection for packaging blood products because a container with a longer shelf life reduces product losses.

1.10 Recent developments in polyolefins and their suitability for replacing PVC

Recent developments in metallocene single-site catalyst technology permit precise control of molecular architecture and enable the production of polyolefin resins with very low densities and narrow molecular-weight distribution (167). Metallocene-catalyzed polyethylene copolymer resins (mPO) are currently being made with specific gravities in the range of 0.86–0.92 and comonomer content of 0–45%. Polyolefin plastomer resins formulated as ethylene-octene copolymers with less than 20% comonomer have demonstrated enhanced toughness, sealability, clarity, and elasticity with no additives and no leachabilitity.

Metallocene Polyolefins

Metallocenes are generally single site catalysts that mean all the catalytic sites of the system are identical. Due to its advantages of the single-site, metallocene catalysts produce very uniform homo- and copolymers with narrow molecular weight distribution, controlled stereoregularities and regioregularities, and random incorporation of comonomers such as 1-olefins (168). It has become an important technology in the global polymer industry for the polymerization of polyolefins.

Metallocene is a coordination compound consisting of a transition metal ion, such as zirconium or titanium, with one or two cyclopentadienyl ligands. The ligands are frequently joined by a "short bridge" that constrains the shape of the complex to a "clam shell" geometry. The discovery that opened the door to commercial success was that substitution of the cyclopentadienyl rings allowing the metallocene to produce high molecular weight polymers. The length and structure of the bridge and the nature of the cyclopentadienyl ring substitution are critical factors that control the activity and selectivity of the catalyst, the structure, and sometimes the stereochemistry of the final product. The structures of the two typical metallocene catalysts are shown in Fig. 1.4

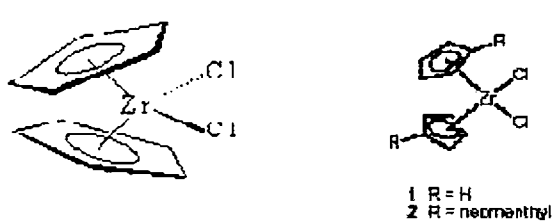

Figure 1.4 Structure of two typical Metallocene catalysts

Ernst O. Fischer and GeoVrey Wilkinson discovered the structure of metallocenes, so called 'sandwich compounds' in which a p-bonded metal atom is situated between two aromatic ring systems, in 1952 (169,170). They were both awarded the Nobel Prize in 1973 for this achievement. This compound class initiated a more resourceful organometallic chemistry that did not play a role in larger industrial processes in the past. Metallocenes, in combination with the conventional aluminium alkyl cocatalysts used in Ziegler systems, are indeed capable of polymerizing ethane, but only at a very low activity. It was found later that small amounts of water were

found to increase significantly the activity of the catalyst (171). The reaction between water and aluminium alkyl was shown to produce alkyl aluminoxane. Only with the discovery and application of methylaluminoxane (MAO) as a co catalyst enhanced the activity, surprisingly, by a factor of 10 000 (172-173). Therefore, MAO plays a crucial part in catalysis with metallocenes. Methylaluminoxane is a compound in which aluminium and oxygen atoms are arranged alternately and free valences are saturated by methyl substituents. It is gained by careful partial hydrolysis of trimethylaluminium and, according to investigations by Sinn (174) and Barron, (175) it consists mainly of units of the basic structure $[Al_4O_3Me_6]$, which contains four aluminium, three oxygen atoms and six methyl groups. As the aluminium atoms in this structure are coordinatively unsaturated, the basic units (mostly four) join together forming clusters and cages shape as depicted in the figure 1.5.

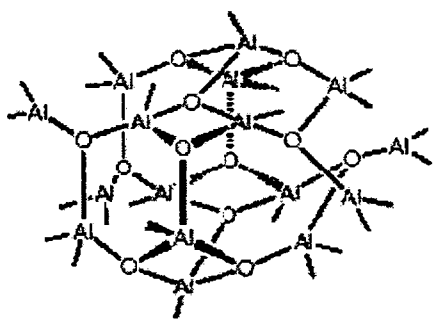

Figure 1.5 Structure of Methylaluminoxane cocatalyst

Many researches have been done on metallocene catalyst and the comparison of Ziegler-Natta and metallocene based polyolefin were made into several ways.

In comparison with Zeigler–Natta catalyst, metallocene catalysts represent a great development: they are soluble in hydrocarbons, show only one type of active site and their chemical structure can be easily changed. These properties allow one to predict accurately the properties of the resulting polyolefins by knowing the structure of the catalyst used during their manufacture and to control the resulting molecular weight and distribution, comonomer content and tacticity by careful selection of the appropriate reactor conditions. In addition, their catalytic activity is 10–100 times higher than that of the classic Zeigler–Natta systems.

Polyolefins, with different microstructures and characteristics, can be custom-made just by varying the ligands on the metallocene (176-182). Metallocene catalysts produce polymers with polydispersity (Mw/Mn) approaching 2 whereas Ziegler-Natta catalysts frequently yield Mw/Mn equal to 5.

The mechanism of olefin polymerization by zirconocenes is as shown in the figure 1.6. Initially the co catalyst converts the catalyst zirconocene into a active specie which has a free co-ordination position for the monomer and stabilizes the latter. Then the olefin monomer is inserted into the zirconium-alkyl bond and provides a new free co-ordination position for another monomer unit. The final step is repeated and thus rendering a polymer chain. This whole process is taking place in a very short period of time (about 2000 olefin molecules per catalyst molecule per second)

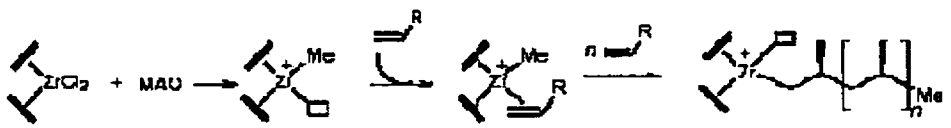

Figure 1.6 Polymerization mechanism of Olefins by Zirconocenes

The historical events of the evolution of the metallocene catalyst structures for olefin polymerization are shown in Table 1.5.

Table 1.5. Time table and historical events of metallocene research

1952	Development of the structure of metallocenes (ferrocene) by Fischer and Wilkinson
1955	Metallocene as component of Ziegler-Natta catalysts, low activity with common aluminium alkyls.
1973	Addition of small amount of water to increase the activity (Al:H2O = 1:0.05 up to 1:0.3) (Reichert, Meyer and Breslow)
1975	Unusual increase in activity by adding water at the ratio Al:H2O = 1:2 (Kaminsky, Sinn and Motweiler)
1977	Using separately prepared methylaluminoxane (MAO) as cocatalyst for olefin polymerization. (Kaminsky and Sinn)
1982	Synthesis of ansa metallocenes with C2 symmetry (Brintzinger)
1984	Polymerization of propylene using a rac/meso mixture of ansa titanocenes lead to partially isotactic polypropylene. (Ewen)
1984	Chiral ansa zirconocenes produce highly isotactic polypropylene (Kaminsky and Brintzinger

Metallocene polyethylenes (mPE) usually have improved toughness and better optical properties compared to conventional polyethylenes. The narrow MWD provides good physical properties to mPE) (183-184). However mPEs tend to have more warpage. The narrow MWD of mPE makes them less sensitive to shear rates. The decreased shear thinning at the time of processing

demands higher energy requirements for processing and the low level of shear-thinning results in processing difficulties, such as sharkskin in extrusion. These processing difficulties can be overcome by the incorporation of short and long chain branching (185). In an effort to improve on the processing performance of m-PE's, researchers at Dow Chemical Company (186) have indicated that branching can be incorporated into m-PE's by forming copolymers containing an alpha-olefin such as octene. This type of branching can be obtained by using a special type constrained geometry metallocene catalyst. Dow Chemical Company has commercialized the catalyst and the process technology under the tradename INSITE. The long chain branging in the Dow's metallocene PE (Affinity) increase the amount of shear thinning, increase melt strength and reduce susceptibility to melt fracture and draw resonance (187) and therefore improve their processability. In constrained geometry catalyst one cyclopentadienyl ring has been replaced by a heteroatom (often N) attached to a bridging atom as in the figure 1.7. M = transition metal, usually goup 4b (Zr, Ti, Hf); A = optional bridging atom, generally Si or C atom with R=CH3; R = H, alkyl, or other hydrocarbon groups, which need not all be identical; X=halogen atom (generally CI) or alkyl group. The characteristic feature of the structure of the constrained geometry catalyst is the short angle (less than 115°) between Cp, M and N atoms instead of 120°-140° between the two Cp rings as in the case of ordinary metallocene catalyst (188).

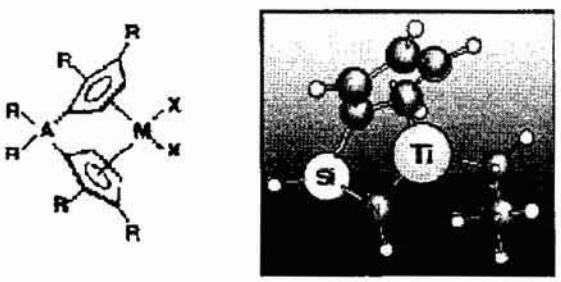

Figure1.7 Structure of Constrained Geometry Metallocene catalyst

The single site constrained geometry catalysts have unusually high activities and thus can be used in small quantities. They also have high activity at higher temperature than ordinary metallocene. It is different from usual metallocene because of its ability to incorporate chain branging into the polymer structure as a result of greater exposure of active metal site.

The polyolefins, especially those based on metallocene technology, are most likely to compete with PVC in the long run because they meet all the criteria critical to PVC (189). The combination of a film density approximately 30% lower than that of PVC and improved properties results in a thinner, lighter-weight product that meets performance needs while reducing the volume of material required—making the product lighter to ship and use and creating a lower volume of waste material for disposable devices. The higher yield can also allow mPO films to be very cost-competitive: although PVC resins cost less per pound than mPO resins, the price per unit area of film or per finished device can be comparable. Metallocene polyolefins also have a lower melt processing temperature, much lower injection molding cycle times (up to 25% less than PVC) and offer potential price savings of 25%-50% (190). Downgauging also reduces disposal costs for hospitals by reducing the tonnage of waste. Finally, metallocene polyolefins can likely be produced using the same equipment as PVC.

With all these attributes, it is anticipated that mPO can provide a high-performance, practical and cost-effective alternative to PVC.

1.11 Modification of Metallocene Polyolefin

The process of selecting a suitable material for a particular medical product needs a precise and accurate understanding of its material and functional requirements (191-192). Instead of developing new polymeric materials, it is the general trend to concentrate on the development of new blends of the existing polymers, which can be tailored to suit various applications and performance characteristics of the product. The blending of polymers to achieve a superior product is used widely in industry (193-197). This type of development is carried out mainly to avoid the time lag and the developmental cost of the material.

Many studies have been reported on the modification of metallocene polyolefins with different polymers to improve the properties of the former. Cran et al. found that even a minimal addition of LLDPE (mPE) to LDPE enhances the crystallization rate and improves various film properties such as impact strength, optical clarity, film toughness and tensile properties (198). Wu and his coworkers used ethylene vinyl acetate copolymer (EVA) to improve the processability of mPE (199-200). Peo'n et al recently demonstrated that the addition of EVA to polyethylene (PE) greatly improves its rheological properties in terms of elasticity (201). Horng-Jer Tai in his study on the EVA/m-PO blend investigated the molecular structure development of EVA and m-POE. In another work Kontopoulou et al used binary blends of EVA and mPE for film applications to improve the processability and heat seal properties of m-PE (202).

Several polymeric blends and alloys of metallocene polyethylenes are in the trail to replace plasticised PVC from many of its industrial as well as medical applications (203-205). In one of such attempts, a miscible alloy of a metallocene based ultra low-density polyethylene with high-density polyethylene was developed to use as an alternatives to PVC for flexible medical bags applications(204). The product was reported to have high clarity, flexibility and easily collapsible inside a protective canister. The HDPE boosted the melt viscosity and softening point of the blend giving it the required processability and durability, which the application requires. In another attempt, radiation –tolerant materials for injection –molded medical devices was developed using the blend of metallocene-catalysed ethylene-based plastomer with propylene homopolymer, which can be used to give films with the desired improved resistance to embrittlement after Y irradiation (206,207). But all these studies were discussing only the processability, mechanical properties and clarity of the films produced with these alloys and blends. A complete evaluation like processability, mechanical properties, clarity, surface properties, permeability and biological especially the blood compatibility of the material was not done on those modified materials. So a complete evaluation of the modified material with respect to processability, mechanical properties, clarity, surface properties, permeability and blood compatibility is worth studying.

1.12 Scope and Objectives of the Present Study

In considering the population health effects of a product's use, one must consider the potential effects of the product throughout its life cycle, from production, through use, to disposal. During both its production and disposal, PVC is associated with the production of many toxic effluents like vinyl chloride, dioxins, hydrochloric acid etc. Many studies indicated that these toxic emissions adversely affect the environment, wildlife and mankind.

The review of the literature also suggests that humans are exposed to substantial levels of DEHP through PVC medical devices. Based on scientific studies in animals and cell cultures and some limited studies in humans, this exposure may lead to adverse health effects particularly to long-term medical care patients such as hemophiliacs and dialysis patients, as well as neonates and the developing fetus.

Therefore, a precautionary approach should be applied to minimize the risk to humans from exposure to DEHP through medical devices and exposure to the toxic effluents produced at the time of production and disposal of PVC products.

Review of the literature suggests that PVC alternatives are widely available for use in most medical devices and may be cost-competitive. Several U.S. and European medical device manufacturers have already developed PVC-free alternatives for IV bags, tubing, and platelet storage.

Presently, PVC occupies a strong position in the red blood cells packaging bags, and tubing industry. A suitable alternative to PVC is not currently available in the market for packaging red blood cells. Many research and development programmes are going on in this direction to find a suitable non-PVC material for red blood cells storage applications. But a material that offers the material advantages of PVC used for the storage of red blood cells is yet to be identified. Additional efforts are needed to find out a true material that matches all the attributes of PVC especially in the flexible medical applications.

Considering the growing concerns about DEHP and the PVC life cycle, any approach in the development and successful production of a PVC-free alternative for red blood cell storage would be at a unique advantage from the environmental as well as the human health point of view.

Recent developments in metallocene single-site catalyst technology produced a new class of polyolefins having improved performance properties like enhanced toughness, sealability, clarity, and elasticity. These materials have a high potential as replacements for flexible PVC in the coming years. The combination of a film density approximately 30% lower than that of PVC and improved properties results in a thinner, lighter-weight product that meets performance needs while reducing the volume of material required—making the product lighter for transportation and use and creating a lower volume of waste material from disposable devices. The higher yield can also allow mPO films to be very cost-competitive: although PVC resins cost less per pound than mPO resins, the price per unit area of film or per finished device can be comparable.

So, it is of utmost importance to identify a safer plasticizer in place of DEHP or find an alternative material to PVC for blood and blood component storage applications. This study has been undertaken from this perspective.

The specific objectives of this study are,

▮ Use of elastomeric plasticizers as partial replacement of DEHP

It is proposed to use high molecular weight elastomers such as acrylonitrile butadiene rubber(-NBR), epoxidized natural rubber(ENR) and carboxylated nitrile rubber(XNBR) as polymeric plasticizers in place of DEHP. The salient features of these modified materials are proposed to be compared with those of DEHP plasticized PVC.

▮ Use of novel metallocene polyolefin as total replacement of plasticized PVC.

It is proposed to replace plasticised PVC completely with the novel class of metallocene polyolefin. The suitability of this material for biomedical applications is proposed to be evaluated in comparison to pPVC.

▮ Use of modified metallocene polyolefin for specific applications

It is proposed to modify metallocene polyolefin with selected polymers such as ethylene vinyl acetate copolymers to improve specific properties such as gas permeability and blood compatibility. The effect of sterilization on mechanical properties as well as gas permeability of the modified materials is proposed to be evaluated. Evaluation of the cytotoxicity of the virgin as well as the modified samples is proposed to be carried out. A comprehensive blood compatibility study which includes tests such as clotting time, cell adhesion and haemolysis of the most potential materials is also proposed to be undertaken.

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Chapter 2 MATERIALS AND METHODS

2

MATERIALS AND METHODS

2.1 Raw Materials

In the first phase of this study, poly (vinyl chloride) (PVC) was used as the base material. Three types of elastomers were used as polymeric plasticizers for PVC. In the second phase a metallocene based polyethylene-octene copolymer (mPO) having 9.5% comonomer content was the base material. Two grades of ethylene vinyl acetate co-polymers (EVA) having different vinyl acetate content were used for the modification of mPO.

2.1.1 Poly (vinyl chloride)

Poly (vinyl chloride) was procured from Reliance India Limited Mumbai, India. The polymer was obtained in the form of free flowing powder. The specifications of the grade chosen for the study and other details of the polymer as per the company's literature are given in table 2.1.

Table 2.1 PVC resin Specification

Property	*Values*
Grade	Reon 6701
K-value	67 ± 1
Heat loss	0.3% max
Porosity (DOP Absorption)	0.19-0.27ml/g
Bulk density	0.53-0.59 g/ml
Residual VCA	1ppm max
Dark resin count	4 No. max.
Fish eye count	20 No. max.
Flow time	25 s max.
Electrical conductivity	< 6 □mhos /cm/g

2.1.2 Additives for PVC

The following chemicals were used as plasticizers and stabilizer for compounding PVC. All these chemicals were obtained from Hindustan Latex Limited, Thiruvananthapuram, Kerala, India.

a) Diethyl hexyl phthalate (DEHP)

b) Di-octyl adipate (DOA)

c) Epoxidised oil

d) Ca-Zn stabilizer

e) Phosphite chelator.

The physical properties of DEHP are given in the table 2.2.

Table 2.2 *Physical Properties of DEHP**

Chemical Name	Di(2-ethylhexyl) phthalate
Empirical formula	$C_{24}H_{38}O_4$
Molecular weight	390.57
Melting point	- 46 °C
Boiling point	386 °C
Vapour pressure	4.6 x 10 -5 Pa at 25 °C
Solubility in water	Essentially insoluble: < 3 µg/L at 20 °C

* *data obtained from DEHP Center (net)*

2.1.3 Acrylonitrile butadiene rubber (NBR)

Acrylonitrile butadiene rubber having acrylonitile content 33% was obtained from M/s. Apar Industries India Limited.

2.1.4 Epoxidised natural rubber (ENR)

This elasotomer was received from Guthrie Polymer SDN BDH; Malaysia and the percentage epoxidization of the rubber is 50%

2.1.5 Carboxylated acrylonitrile butadiene rubber (XNBR)

The carboxilated acrylonitrile butadiene having 7.5% carboxilation rubber was also obtained NIPOL 1072, Zeon Chemicals Inc., Japan.

2.1.6 Metallocene based polyolefin plastomer (mPO)

The polyolefin copolymer containing 9.5% octane (Affinity Polyolefin Plastomer PL 1845) polymerized by metallocene single site constrained geometry catalyst was obtained from Dow Plastics, Dow Chemical Company, Midland. The polymer was in the bead form, having 2-3mm size. The specifications of the plastomer are as given in the table 2.2

Table 2.3. Properties of Polyolefin Plastomer (Affinity PL 1845) *

Physical Properties	*Test Method*	*Values*
Percentage comonomer, Octene	Dow, based on ASTM D-2238, Method B	9.5
Melt Index, dg/min	ASTM D-1238	3.5
Density, gm/cc	ASTM D-792	0.910
DSC Melting Point, ^{o}C	Dow	103
Dow Rheology Index (DRI)	Dow	1.4
Puncture Resistance, J/cm3	Dow	18
Dart Impact, g (Method B)	ASTM D-1709	470
Elmendorf Tear Strength, g (Type A)		
MD	ASTM D-1922	178
CD	ASTM D-1922	362
Ultimate Tensile (MPa)		
MD	ASTM D-882	45.4
CD	ASTM D-882	33.4
Ultimate Elongation, %		
MD	ASTM D-882	527
CD	ASTM D-882	664
Clarity	ASTM D-1746	75
Gloss, 20^{o}	ASTM D-2457	145
Haze, %	ASTM D-1003	0.7
Seal Initiation Temperature, °C	Dow	99

* Data taken from manufacturer's product data sheet. As per the manufacturer, Affinity PL 1845 polymer complies with FDA regulation 21 CFR 177.1520 (c) 3.2a, which allows for its use in direct food contact applications.

2.1.7 Ethylene vinyl acetate co-polymer

Two grades of EVA copolymer with different vinyl acetate content i.e., 12 and 18 were procured from National Organic Chemicals India Limited, Mumbai, India. The copolymers were obtained in the form of beads, having 2-3mm size.

Table 2.4. Properties of ethylene vinyl acetate co-polymers (EVA)

Polymer Grade	*EVA12*	*EVA18*
Vinyl acetate content, (wt.%)#	12	18
Melt flow index, {/g (10 min-1)}#	2	10
Density, g/cc#	0.931	0.937
Melting point, °C#	95	88

Data taken from manufacturer's product data sheet

2.2 Preperation of Blends

2.2.1 PVC/Elastomer Blends

PVC was mixed with other ingredients as per Table 2.5, except the polymeric plasticizer, by the dry blending technique in high-speed mixer. Blends of PVC and three different elastomers with varying compositions as mentioned in table 2.5 were prepared by mixing in a Haake Torque rheometer (Model: Rheomix-600) fitted with cam rotors at 160°C using a rotor speed of 40rpm. Compounded PVC was initially melted in the mixer for 1-2 minutes and then masticated rubber strips were added to molten PVC till the mixing torque was stabilized. The blend thus obtained was passed through a laboratory two-roll mill set at 2 mm-nip setting to get a sheet, which was subsequently compression moulded between aluminium foils at 160°C for 3 minutes at a pressure of 100 kg/cm2 in an electrically heated press (Santhosh Industries, India) to obtain sheets of dimensions120mm x 120mm x 0.3mm.

Table 2.5: Composition* of control PVC and modified PVC with different polymeric plasticizers (NBR,

Material	*A (Control)*	*B*	*C*	*D*	*E*	*F*
PVC	100	100	100	100	100	100
Polymeric plasticizer (NBR or XNBR or ENR)	-	7.5	15	20	25	25
DEHP	40	32.5	25	20	15	-
Di-octyl adipate	10	10	10	10	10	-
Epoxidised oil	7	7	7	7	7	-
Ca-Zn stabilizer	2.5	2.5	2.5	2.5	2.5	-
Phosphite chelator	0.5	0.5	0.5	0.5	0.5	-

Torque rheometer

The torque rheometer consists of horizontally mounted, heavy-duty motor drive together with a torque sensor. The basic components of the rheometer are a motor, a torque sensor and a mixing head. Within the mixing head are the rotors that rotate at different speeds within adjoining circular cavities. The mixing head consists of a steel cell in which the sample fills a 'bowl' consisting of two adjoining cylindrical chambers. This cell may be heated either by circulating thermal fluid or by electrical heating elements. Once mixing is begun considerable dissipative heat can be generated, and it becomes necessary to remove the heat. Thermocouples are mounted in the mixing head to measure the temperature of the melt. While carrying out a process-ability test, the torque required to stir a molten polymer in the mixing chamber is measured as a function of time. The Rheomix 600 is the standard internal, intensive mixer used for a wide range of applications, particularly for testing thermoplastics (figure 2.1). The three section mixing chamber is electrically heated and air cooled, thus enabling simple and rapid testing and cleaning.

Figure 2.1 Photograph of the Haake Rheomix 600

2.2.2 mPO/EVA Blends

The different compositions of mPO /EVA produced along with their designations are given in Table 2.6.

The blends of mPO /EVA were prepared by mixing in a Brabender Torque Plastograph (Model: Rheomix-600) fitted with cam rotors at 160^{o}C using a rotor speed of 40rpm. for 6 min. At the end of 6 min, the blends were dumped out of the internal mixer and compression moulded in a laboratory model hydraulic press (Santhosh Industries, Mumbai). The temperature and pressure used for the compression moulding were 170^{o}C and 100 kg/sq. cm, respectively.

Table 2.6 Summary of test materials of mPO/EVA blends

Material Code	*Polymer composition (wt%)*		
	mPO	*EVA12*	*EVA18*
mPO	100	-	-
AE1205	95	05	-
AE1215	85	15	-
AE1225	75	25	-
AE1250	50	50	-
EVA12	-	100	-
AE1805	95	-	05
AE1815	85	-	15
AE1825	75	-	25
AE1850	50	-	50
EVA18	-	-	100

2.3 Test Procedures

After mixing and moulding, the blends were subjected to the following tests

2.3.1 Mechanical property evaluation

2.3.1.1 Tensile properties

The tensile mechanical properties were measured using universal testing machine (Instron model No.3345) with 100 N load cell and a constant crosshead speed of 100 mm/min. at 22 + 2°C. The test was conducted according to the ASTM 638-03 (1). The specimen dimensions were in accordance with ASTM specification. The strain rate employed for testing was 1min-1. Mean values were derived from tests performed on six dumbbell specimens.

2.3.1.2 Tear strength

This test describes the procedure for measuring tear strength of samples and was carried out as per the ASTM designation D624-98 (2). Five specimens were cut out from the moulded sheet with the help of a steel die (Die C) as depicted in the figure 2.2. The thicknesses of the specimen were measured in three places across the width of the specimen near its center with a micrometer. The samples were conditioned for 18 hours at 23 + 2°C. After conditioning the samples were mounted in the tensile test machine (Instron model No.3345). A steady increasing traction force was applied at a rate of separation of the grip 500 mm/min and the tear strength was calculated using the formula

$$TS = F/d \text{ ----------(2.1)}$$

where Ts is the tear strength, F the force applied and d the thickness of the test specimen and expressed in kN/m.

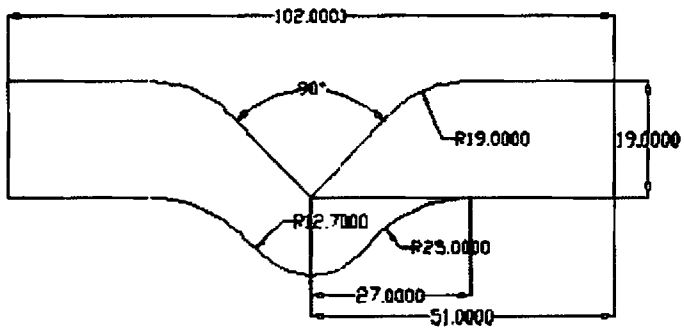

Figure 2.2 Drawing of Die C (all measurements are in mm)

2.3.2 Thermal Analysis

2.3.2.1 Thermo Gravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) is used to investigate thermal stability of the material. TGA Q 50 (TA Instruments) was used at a heating rate of 10°C/min from room temperature to 800°C with 10-12 mg of sample, in a nitrogen atmosphere.

2.3.2.2 Differential Scanning Calorimeter

A DSC was used to measure the transitions in the polymers. The measurement was performed on DSC-2920 (TA instruments, USA), based on ASTM E1356-98 (3). All the samples were dried prior to the measurements and analyses were done in a nitrogen atmosphere using standard aluminum pans. The test method involves continuously monitoring the difference in heat flow into, or temperature between a reference material and a test material when they are heated and cooled at a controlled rate through the glass transition region of the test material and analyzing the resultant thermal curve to provide the glass transition temperature. A heating rate of 10^{O}-C/min was employed and the material was scanned in the temperature range of -50^{O}C to 150^{O}C.

2.3.3 Leaching Studies:

Migration of the pastizicer from control (plasticized PVC) as well as other modified samples was carried out in n-hexane at three different temperatures 10, 25 and 40 ± 2^{O}C. Samples were weighed and kept in 10mL of n-hexane in different test tubes. Occasional shaking of the tubes was carried out. The samples were taken out at different intervals of time over a period of 72 hours, dried in an air oven and the weights of the dried samples were taken. From the difference in weights, the amount of DEHP migrated into the medium was determined. Values reported are the average of three values.

2.3.4 Density Measurements

The densities of the samples were calculated using a Sartorius precision balance (LA 230S, Sartorius, Germany) with the help of a specific gravity kit. The density (ρ) of the samples were calculated using the formula (Sartorius Manuel):

$$\rho = \frac{W_a \rho_0}{0.99983\,G} + 0.0013 \text{----------} 2.2$$

where W_a is the weight of the material in air, ρ_0 the density of water at the test temperature, 0.99983 the correction factor, G the buoyancy and 0.0012 is the density of air under standard conditions.

2.3.5 Hardness Test

Shore A hardness of the control PVC and NBR modified PVC samples were measured using a durometer, (Blue steel Engineers Pvt. Ltd., India) as per ASTM D2240-97 (4).

2.3.6 Melt Flow Index Measurements

Melt flow index is an important parameter in the flow property, which in turn the processability of polymers. Melt flow index of the samples were measured using MFI tester (Frank Devices, Italy) as per ASTM D1238-04c specification (5). The melt flow index apparatus is preheated to a specified temperature of 190 ± 2°C. The samples were loaded from the top of the cylinder and a load of 2.16 kg was placed on the piston. The material was allowed to extrude through the die. The initial extrudate was discarded as it contains air bubbles or contaminations. The extrudate was cut at a time interval of 60 sec. and was weighed. The melt flow index (MFI) values were expressed in gm/10 min. Melt flow index is an important parameter in the flow property, which in turn the processability of polymers. It is the amount of molten polymer comes out of an orifice having mm diameter at 190^{O}C on applying a load of 2.16 kg load in 10 minutes.

2.3.7 Contact Angle Measurements

The samples were cleaned with 0.5% soap solution (Teepol) in distilled water. After proper rinsing with distilled water (5 times) the samples were dried at 60 ± 2°C in an air oven. Drops of double distilled water (3μl) were deposited onto the cleaned and dried sample surface using a hypodermic syringe. The angle made by the water drop with the sample surface was measured using an optical bench type contact angle goniometer (Rame-Hart, Inc. model 100-0) {Figure 2.3 (a) and (b)} at 23 ± 2°C. The angles on the both sides of the drop were taken and an average of 20 measurements was reported.

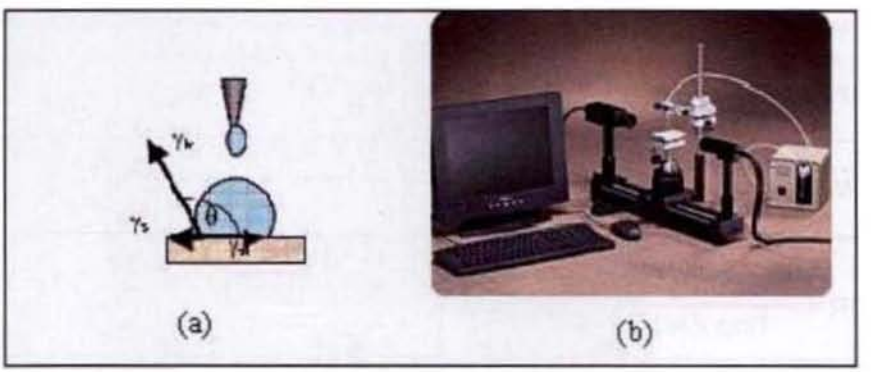

Figure 2.3 (a) Schematic diagram of a contact angle measurement (b) Photograph of a Goniometer

2.3.8 Water Absorption Studies

Initially the samples were cleaned with 0.5% soap solution (Teepol) in distilled water. After proper rinsing with distilled water (5 times) the samples were dried at 60 ± 2°C in an air oven. Dry weights of the samples were taken after complete drying (Wa). Then the samples were immersed in distilled water for 72 hours. The samples were taken out from the water at a specified time interval, wiped out of the water adsorbed on the surface of the samples with the help of filter paper and reweighed immediately (Wb). Percentage of water absorbed by the samples were calculated by the following equation

$$\% \text{ Water absoption} = \frac{(Wb)\,(Wa) \times 100}{(Wa)} \qquad \text{----------(2.3)}$$

2.3.9 Transparency Tests

The attribute of clarity of a sheet, measured by its ability to transmit visible light, correlates with its regular transmittance. The percentage transmittance of the visible light through the cleaned sample sheets having the size of 1x3cm2 and 0.3 mm thickness were determined at 540nm using a UV-Visible spectrophotometer (Shimadzu, model UV-1601) The tests were conducted as per ASTM D1746-97 (6) under the test conditions at 23 ± 2°C and 50 ± 5% relative humidity.

2.3.10Water Permeability Testing

Water vapour transmission rates (WVTR) of the samples for 24 h were measured using an in house setup. The technique used to measure water vapour transmission rate was a modification of the wet cup method described by ASTM E96-95 (7). In this method the test film covered a 100 ml. beaker filled with distilled water. The mass of water lost from the beaker was measured as a function of time and the WVTR was calculated from the steady state region using equations 2 & 3. Thickness of the sample was measured using a thickness gauge at a minimum of 15 positions. The standard deviation of thickness for each specimen was less than 5%. A window of known area was cut from two sheets of aluminium foils and the sample was thoroughly fixed in between them. Then the aluminium foils with the test sample was mounted on the beaker with the help of adhesive tape (Figure 2.4). A control setup was also made without the samples.

$WVTR = \frac{\text{Mass of } H_2O \text{ lost}}{\text{Time x Area}}$	----------(2.4)
$Sp.WVTR = \frac{\text{Mass of } H_2O \text{ lost}}{\text{Time x Area}} \times \text{thickness}$	----------(2.5)

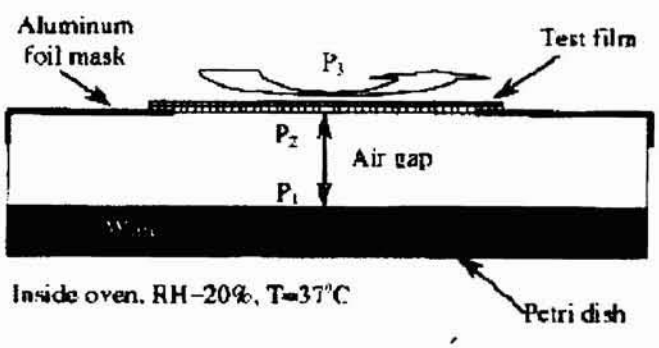

Figure 2.4 Schematic diagram of the setup for WVTR studies

2.3.11 Gas Permeability Studies

The gas permeability measurements were made using samples of 120 mm diameter and 0.3 mm thickness. The samples were preconditioned according to ASTM D618-96 (8). The gas permeability measurements were carried out using oxygen and carbon dioxide in a manometric gas permeability tester (Model L-100, 2402/1, Switzerland)(Figure 2.5) at three different temperatures (10, 25 and 40^{o}C) as per ASTM D1434-98 (9).

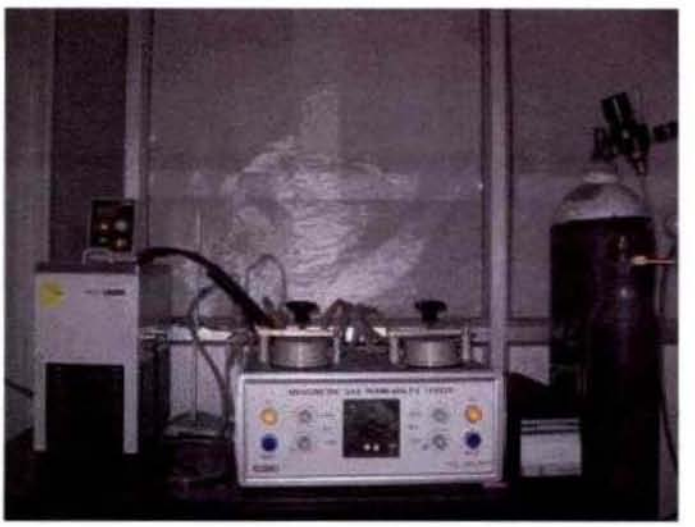

Figure 2.5 Photograph of the Gas permeability tester

2.3.12 Phase Morphology

The cryogenically fractured surfaces of the mPO, EVA12, AE1225 were examined in Scanning electron microscope (JOEL model 4200) at various magnifications. The fractured surfaces of mPO, AE1225 and AE1250 after etching in dichloroethane for 3hours at 70 ± 2^{o}C were also examined to find out the phase distribution of EVA in the blend composition.

2.3.13 Sterilization of the Materials

Some of the samples were sterilized by radiation at a dose of 2.5 Mrad using a source of ^{60}Co (BARC, Mumbai). To find effect of sterilization on the material properties the samples were subjected to mechanical and permeability properties studies after the irradiation.

2.3.14 pH Measurements

To see the possibility of degradation of EVA due to -radiation strilization. The pH of the extract of the samples immersed in deionized water for 72 hours were examined using a pH meter (Cyber Scan 510, Eutech Instruments, Singapore)

2.3.15Biological Evaluation

2.3.15.1 In vitro cell culture cytotoxicity studies

In vitro cell culture cytotoxicity of the samples was assessed as per ISO 10993-5 (1999) (10), using L929 (mouse fibroblast subcutaneous connective tissue) cell line procured from National Centre for Cell Sciences, Pune, India. The cells were maintained in Rosewell Park Memorial Institute (RPMI) 1640 (Himedia, Pune, India) medium supplemented with 10% foetal bovine serum (Sigma, USA) and 100 IU / ml pencillium and 100 µg / ml streptomycine (medical grade). The culture was incubated at 37 ± 2°C in a humidified atmosphere containing 5% carbondioxide with a mediumchange at an interval of 3 days.

2.3.15.1.1 Cytotoxicity – Direct contact test.

Cytotoxicity of NBR modified PVC sample having high NBR content, mPO, and AE1272 by direct contact method was evaluated as per ISO 10993-5 (1999). High-density polyethylene was taken as the negative control and organotin stabilized polyvinyl chloride was used as the positive control and the results were compared. The test is briefly summarized below. A confluent monolayer of L929 mammalian fibroblast cell lines was prepared in a cell culture plate. The culture was incubated as per the conditions mentioned in 2.3.12.1 and examined under a light microscope (Leica DMIL, Germany). The culture medium was removed and replaced with fresh culture medium. Specimens of negative or positive controls and the test article are carefully placed in individually prepared cultures and incubated under the same conditions as in 2.3.12.1. It has to be ensured that the specimen covers approximately one-tenth of the cell layer surface. The culture medium and the specimen were removed and the cell cytotoxicity is evaluated qualitatively. The qualitative evaluation of cytotoxicity involves examining the cells microscopically to assess for changes in general morphology, vacuolization, detachment, cell lysis and membrane. The change from normal cell morphology is recorded as none, slight, moderate and severe, depending upon the extent of cell damage.

2.3.15.1.2 Cytotoxicity – Test on extract

Cytotoxicity of NBR modified PVC sample having high NBR content, mPO, and AE1225 was also evaluated by test on extract as per ISO 10993-5 (1999). Extracts were prepared from the material to simulate the clinical use conditions so as to define the potential toxicological hazard without causing significant changes such as fusion or melting of the material pieces, or alter the chemical structure. The material extracts were prepared by incubating the samples in the culture mediumat 37 ± 2°C for 24 hrs. Phenol and tissue culture grade polystyrene (TCPS) were used as the positive and negative controls, respectively. Culture medium from confluent cells was replaced with material extracts and cytotoxicity was assessed qualitatively after 24 hrs.

2.3.15.2 Blood compatibility studies

The blood compatibility of the modified sample (AE1225) was evaluated by platelet and leukocytes adhesion studies, clotting time tests and hemolysis assay. The results were also compared with that of m-PO and plasticized PVC.

2.3.15.2.1 Platelet and Leukocytes adhesion studies

Blood from human volunter was collected into the anicoagulant, citrate-phosphate-dextrose (CPD). The test materials were placed in polystyrene culture plates and immersed in phosphate buffered saline before they were exposed to blood. To each plate 1.5ml blood was added and a 0.5mL sample was collected immediately for cell count and the remaining 1.0 ml was exposed to the materials for 30 min under agitation at 75±5 rpm using an Environ shaker thermostated at 35±2°C. Three samples were tested for each material. Three empty polystyrene culture dishes were exposed with blood as reference. The count reduction was analysed by detecting the counts in initial and 75 minutes samples using Haematology Analyzer Cobas Minos vet (Roche, France). Total consumption from the exposed blood as the percentage reduction is calculated for each sample.

The materials after 30 minutes exposure were rinsed thoroughly with poshphate buffered saline and were fixed with 1% glutaraldehyde for 1 hour. They were then stained with May Grunwalds stain and viewed under light microscope to detect cell adhesion.

2.3.15.2.2 Haemolysis Assay

The interaction of the material with red blood cells was evaluated using a haemolysis assay as per ASTM F 756-93 (11). Blood (0.2mL) anticoagulated with acid citrarte dextrose was added to 12.5ml of phosphate buffered saline, PBS, containing 0.5g of the samples. Separate positive (100% hemolysis induced by replacing the PBS with 12.5ml of 0.1% sodium carbonate solution) and negative (0% hemolysis, PBS with no material added) controls were also set up. Each set of experiments was repeated 3 times. Samples were incubated for 60 min at 37°C. After incubation the tubes were centrifuged at 3000rpm for 5 minutes. The percentage hemolysis was calculated by measuring the optical density of the supernatant solution at 545 nm spectrophotometrically.

2.3.15.2.3 Clotting Time Test

The kinetics of thrombus formation was determined using fresh human blood according to Xianghuai et al. (12). Samples sheets of pPVC, EVA 18, mPO, AE1225 and AE1825 having size 4cmx4cm were placed on different watch glasses. Fresh human blood (0.1) was placed directly onto the surface of these sheets. After a predetermined time, the specimen was transferred into a beaker containing 50ml of distilled water. The red blood cells, which had not been trapped in thrombi, were haemolysed, and the free hemoglobin in the water was measured using a UV-Visible spectrophotometer (Shimadzu, model UV-1601) at 540 nm.

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Chapter 3 USE OF POLYMERIC PLASTICIZERS IN PVC TO REDUCE DEHP LEACHING

3

USE OF POLYMERIC PLASTICIZERS IN PVC TO REDUCE DEHP LEACHING

3.1 Introduction

Being a rigid polymer, PVC must be compounded with plasticizing agents for several of its applications. These plasticizing agents or plasticizers are organic compounds added to PVC to facilitate processing and increase flexibility and toughness in the final product. Usually, a plasticiser acts as a molecular lubricant in the polymer matrix.

There are several different types of plasticisers that can be used in PVC used for medical application. These include Di-2-ethylhexyl Adipate (DOA), Di-2-ethylhexyl Phthalate (DEHP), Acetyl tri-n-hexyl Citrate (ATHC), n-Butyryl tri-n-hexyl Citrate (BTHC), or Tri-2-ethylhexyl Trimellitate (TEHTM or TOTM). These vary in their characteristics and performance, each having relative advantages and disadvantages under different circumstances. Of these esters, DEHP is the most commonly used plasticizer in medical field. (1-2). Migration of plasticizers is a serious problem in soft PVC especially when used for medical applications (3-5). As plasticizers are generally liquid materials and not strongly linked to the polymer, they can escape from the polymer matrix due to their volatility during storage or by leaching to the surrounding medium. In use loss of plasticizer becomes a very serious concern from two angles (6-7). First, the mechanical properties of polymer article such as abrasion resistance, compressive and tensile strength change considerably with the progressive loss of plasticizer. In addition, flexibility and transparency of polymers are also impared which may reduce them useless in medical applications. Second, extracted plasticizer may contaminate the surrounding medium. Leachability of the additives into the fluid, whether solution or blood, increases with decreasing molecular weight molecular weight of the plasticizer. When PVC is to be in contact with fluids of high extracting power, polymeric plasticizers instead of liquid plasticizers may be more advantageous to check the plasticizer loss due to leaching. Moreover, polymeric plasticizers offer very low volatility, high resistance to extraction, low leachability at elevated temperatures.

Polymer modification by blending two or more different polymers to obtain desired properties is now a common practice. The blending of two or more structurally different polymers provides a convenient and economical route to obtain a polymeric material with tailor-made properties to meet specific needs (8). Blending of PVC with other polymers is used mainly to improve the properties of PVC. Braun indicated that some aliphatic polyesters are compatible with PVC and can be used as polymeric plasticizers (9)

This chapter deals with the attempt made to alleviate the problem of plasticizer migration from plasticized PVC (PPVC) by partially replacing the plasticizer by polymeric plasticizers. Three elastomers, which were known to have good compatibility with PVC, viz, nitrile rubber (NBR) (10-12), carboxylated nitrile rubber (XNBR) (13-15) and epoxidised natural rubber (ENR)(16-18) have been selected for the study as polymeric plasticizer.

Of these three elastomers NBR is reported to have good compatibility with PVC (19-20). Being miscible with PVC and uniformly dispersed in PVC phase, NBR was used as a compatibilizer in many applications (21). Alloys of NBR with plasticized PVC were described as thermoplastic elastomers because they combine ease of melt processing with flexibility and rubber elasticity (22). These alloys show better low temperature flexibility and improved abrasion resistance, which are the two prime properties of the materials used for blood and blood component storage applications (23). Further NBR is also regarded as one of the commercially available elastomers that can be safely used in biomedical applications, generally in in vitro situations. Binary polyblends of plasticized PVC and NBR have been studied for medical applications (24). Thus it is proposed to give more attention to the PVC/NBR system in this study. Gas and water permeability, the properties that are essential for a material to be used for blood component storage application are proposed to be studied in detail. The temperature dependence of permeability as well as DEHP leaching is also proposed to be addressed in this study. The preliminary toxicity evaluation of the system having higher NBR content is also proposed to be carried out by cytotoxicity tests using direct contact and test on extract methods. Since the material is intended for blood contact applications, the behavior of the material towards blood is proposed to be evaluated by clotting time test

3.2 Experimental

Plasticized PVC and the elastomers were melt mixed in a torque Rheometer (Thermo Haake Rheocord 600) at 160°C using cam rotors with a rotor speed of 40rpm for 6 minutes. The torque values of mixing were monitored. The tensile properties of the samples were determined using dumb-bell shaped samples on a universal testing machine (Instron) at a crosshead speed of 100mm/min according to ASTM D638-03. Water vapour transmission rates (WVTR) of the samples for 24 h were measured according to ASTM E96-95. The oxygen and carbon dioxide gas permeability measurements were done in a manometric gas permeability tester at three different temperatures (10, 25 and 40°C) as per ASTM D1434-98. The preliminary toxicity of the blend having maximum NBR content was evaluated by conducting the cytotoxicity test using L929 mouse fibroblasts cell line as per ISO10993 test methods.

3.3 Results and discussion

3.3.1 Effect of Polymeric Plasticizers on Mixing Torque

PVC was mixed with conventional plasticizer and stabilizer as per the formulation given in Table No 2.5 in Chapter 2 in a high-speed mixer using the dry blending technique. Then the PVC compound was mixed with the three elastomers as polymeric plasticizers. The torques vs. time of mixing curves of PVC/elastomer blends; PVC/NBR, PVC/XNBR and PVC/ENR are shown in Figures 3.1-3.3 respectively. Two stage-loading peaks are seen in the figures as expected. The first peak corresponding to that of the melting of PVC and the second one is due to the addition of the elastomer. It may be observed that PVC melts and homogenises in about two minutes and after the addition of the elastomer, the torque stabilizes in about another 4 minutes.

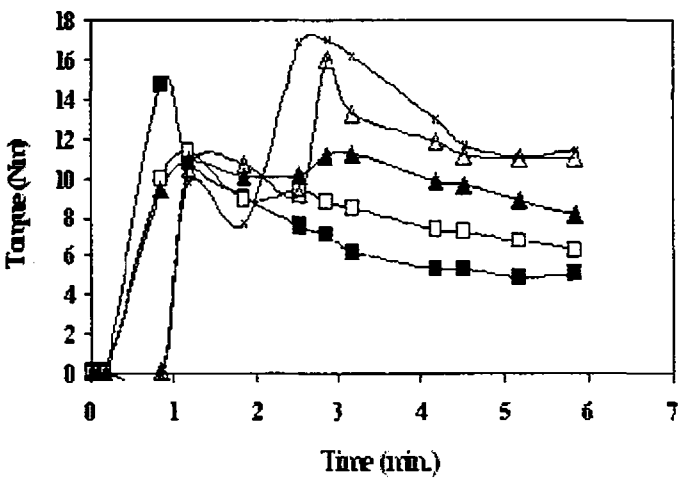


Figure 3.1 Torque vs Time plots for PVC/NBR system containing different NBR contents. ■ Control PVC, ⊐ 7.5phr NBR, ▲ 15phr NBR, Δ 20phrNBR and X 25phrNBR.

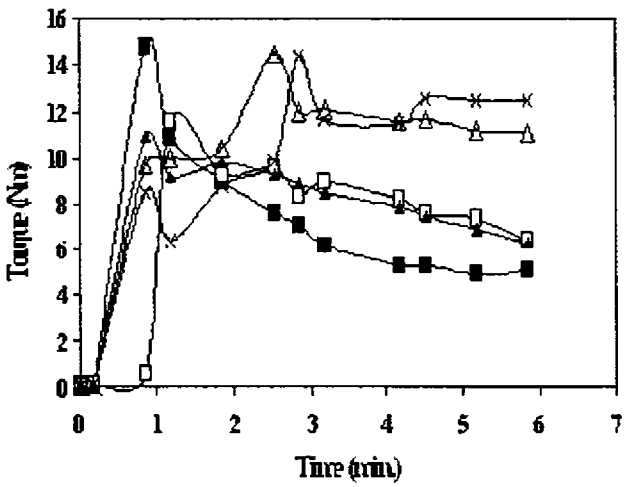


Figure.3.2 Torque vs Time plots for PVC/XNBR system containing different XNBR contents. ■ Control PVC, □ 7.5phr XNBR, ▲15phr XNBR, Δ 20phr XNBR and X 25phr XNBR.

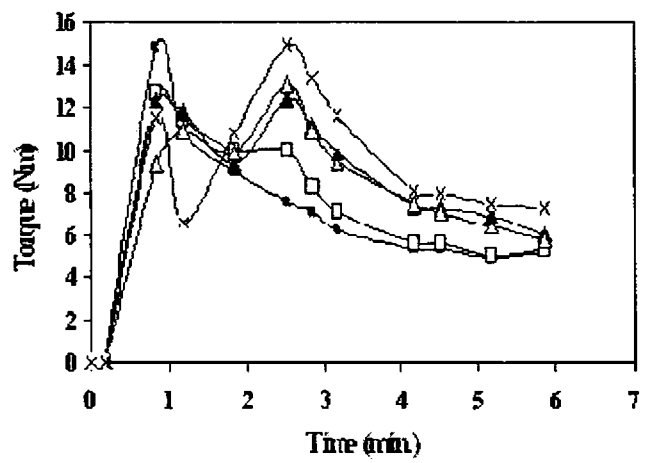


Figure.3. 3 Torque vs Time plots for PVC/ENR system containing different ENR contents. ■ Control PVC, □ 7.5phr ENR, ▲ 15phr ENR, Δ 20phr ENR and X 25phr ENR.

In all cases, an increased torque is observed with the increase of elastomer content. Since the clarity of the film was affected considerably when higher amounts DEHP was replaced by elastomers, only up to 25phr (out of 40phr) DEHP was replaced by the various elastomers. The mixing torque is less in all ratios of PVC/ENR system, compared to the other two systems, which indicate a lower interaction between the polymers.

Figure 3.4 shows the effect of the type of the elastomer in the system on the mixing torque. High mixing torque for XNBR compared to other two elastomers can be seen in the figure. The mixing torque for NBR and ENR are more or less equal. It low initial torque for ENR at the time of the elastomer loading shows the high melting of ENR.

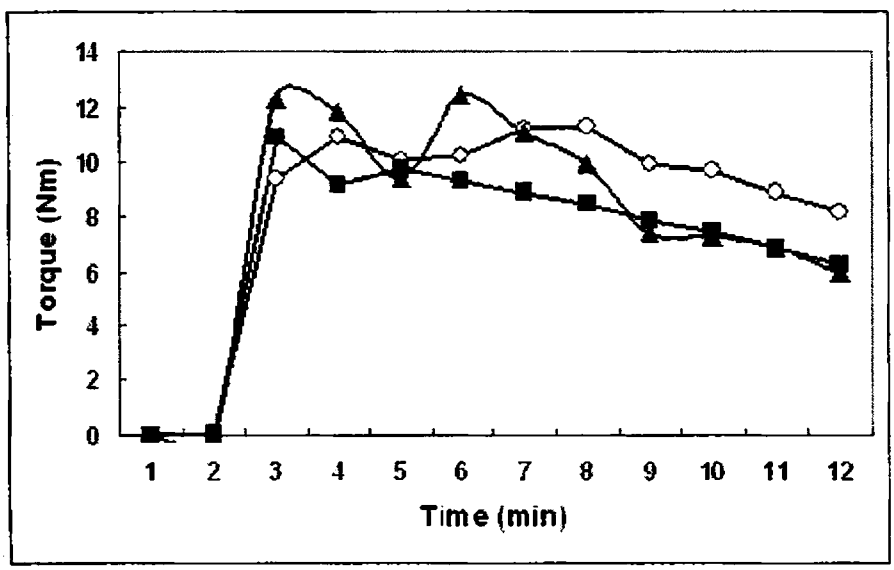


Figure.3. 4 Effect of the type of the Elastomer on Mixing Torque for PVC/NBR, PVC/ENR and PVC/XNBRsystem containing 15phr elastomer content. ▲NBR, ■ ENR and ○-XNBR

3.3.2 Effect of polymeric plasticizers on mechanical properties

The variations of tensile strength of the three systems with different elastomer contents and type of the elastomers are shown in Figure 3.5. In the case of PVC/NBR system, the tensile strength remains more or less the same with the addition of elastomers. But in case of PVC/ENR samples, at 7.5phr ENR, the tensile strength reduces a little and thereafter increases marginally. A sharp decrease in the tensile strength is observed in case of PVC/XNBR samples up to 15phr incorporation, but beyond that an increasing trend is observed. So PVC/NBR system shows the most stable behaviour in tensile strength.

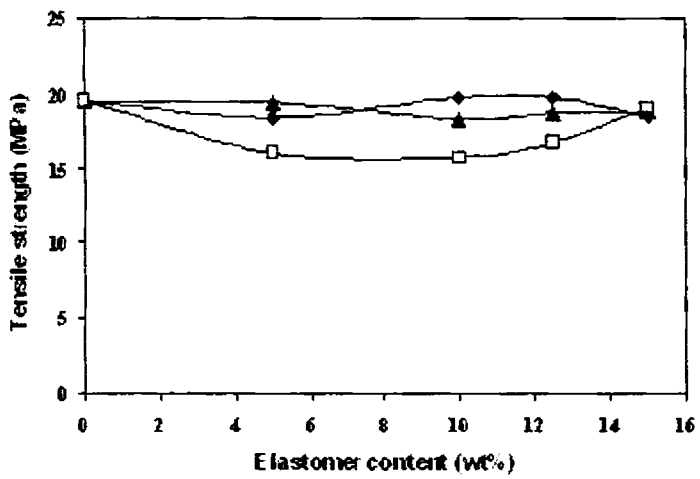


Figure.3. 5 Dependence of tensile strength on percentage content of elastomer for the PVC/elastomer systems. ▲NBR, □ XNBR, and ♦ENR.

Strain at maximum load vs the percentage elastomer content is given in Figure 3.6. In PVC/-XNBR system shows the least value in this case also compared to PVC/NBR and PVC/ENR systems. So from the tensile data PVC/NBR and PVC/ENR may be considered to give more stable behaviour in comparison to PVC/XNBR.

The Young's moduli of the samples with different percentage of elastomer contents are shown in Figure 3.7. PVC/ENR and PVC/XNBR show an increasing trend after 7.5phr elastomer content. In the case of NBR modified PVC samples, moduli are seen very close to that of the control PVC at different NBR contents. This further shows the better compatibility and plasticizing capability of NBR compared to ENR and XNBR.

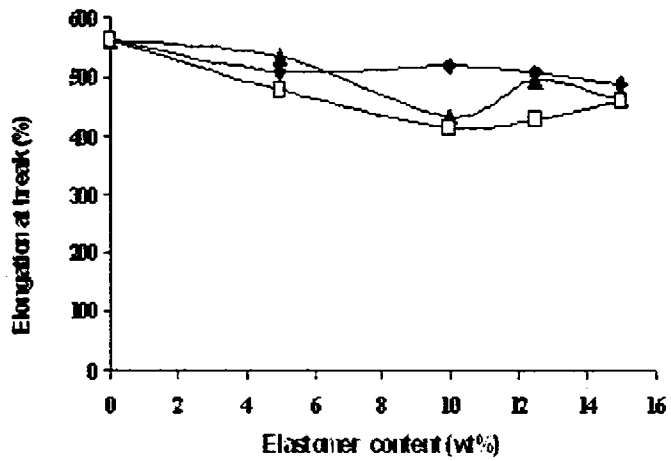


Figure.3. 6 Dependence of elongation at break on percentage content of elastomer for the PVC/elastomer systems. ▲NBR, □ XNBR and ♦ENR.

Stress at different percentage strains of PVC/NBR, PVC/XNBR and PVC/ENR systems are shown in Figure 3.8-3.10 respectively. It may be observed that NBR shows the most stable behavior in this case also. All the curves of the different PVC/NBR blend systems are very close to the control and this shows that the variation of the moduli are very minimal in these cases compared to the other two systems.

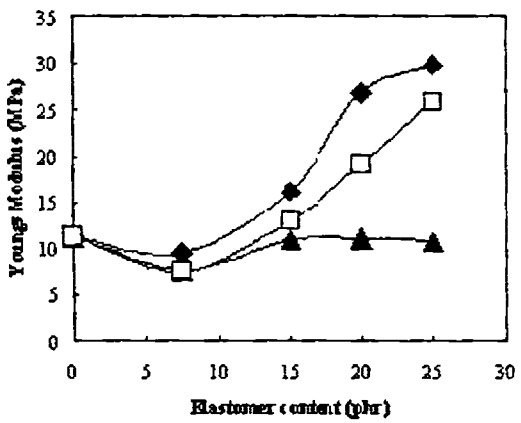
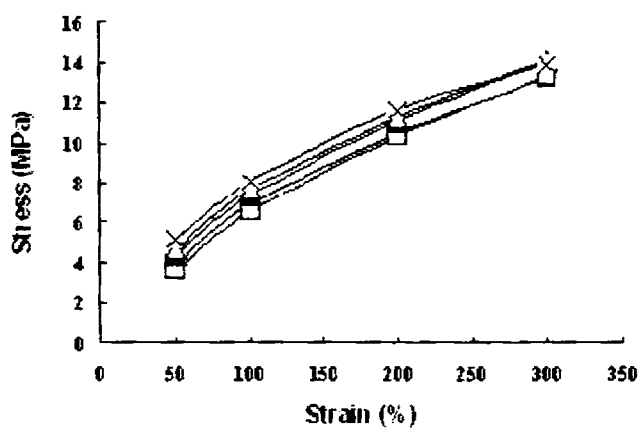


Figure 3.7 Effect of elastomer content in the system on young modulus



Figuve.3. 8 Modulus vs percentage strain plots for PVC/NBR system containing different NBR contents. ■ Control PVC, □ 7.5phr NBR, ▲15phr NBR, Δ 20phrNBR and X 25phr NBR.

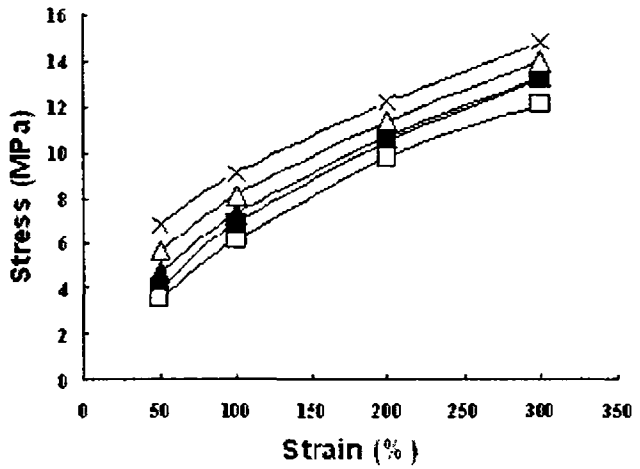


Figure.3. 9 Modulus vs percentage strain plots for PVC/XNBR system containing different XNBR contents. ■ Control PVC, □ 7.5phr XNBR, ▲15phr XNBR, Δ 20phr XNBR and X 25phr XNBR.

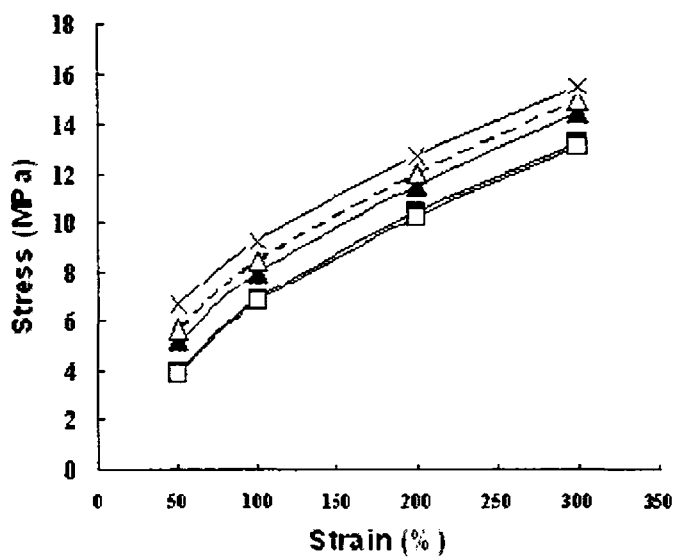


Figure.3. 10 Modulus vs percentage strain plots for PVC/ENR system containing different ENR contents. ■ Control PVC, □ 7.5phr ENR, ▲15phr ENR, Δ 20phr ENR and X 25phr ENR

3.3.3 DEHP Leaching Studies

3.3.3.1 Effect of Elastomer Content

The effect of amount of the elastomer content in the three PVC/elastomers systems for three elastomers, NBR, XNBR and ENR, are seen in the figures 3.11-3.13 respectively. It can be seen that the release of DEHP from PVC plasticized with DEHP alone is very fast and it reaches the maximum level in about 7.5 hours. Similarly, in all the three systems containing a low percentage of polymeric plasticizer (7.5phr), the leaching of DEHP reaches to the maximum level at 7.5 hours. But when the rubber content is progressively increased to 15, 20 and 25phrs, the resistance to the leaching of DEHP increases and varies with the nature of the synthetic rubber. When comparing the tree types of the elastomers on leaching, it can be seen that for the PVC/-NBR system the DEHP leaching is slow and reaches the maximum level only in 48 hours. But in case of ENR and XNBR modified samples the maximum level of DEHP leaching is reached in 24 hours. However, the amount of DEHP leached is less in case of ENR compared to XNBR. The leaching trend for 20phr rubber content is XNBR>ENR>NBR. But at 25phr rubber content the leaching trend is more or less same for ENR and NBR systems.

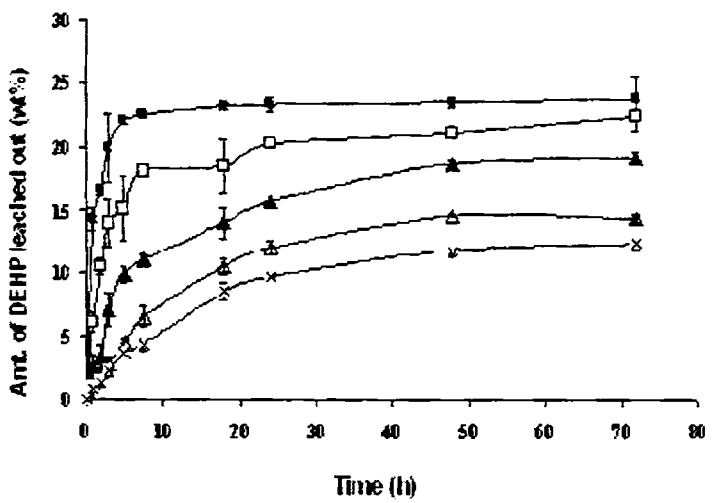


Figure.3. 11 Migration of DEHP from PVC/NBR systems containing different NBR contents. ■ Control PVC, □ 7.5phr NBR, ▲ 15phr NBR, Δ 20phrNBR and X 25phr NBR..

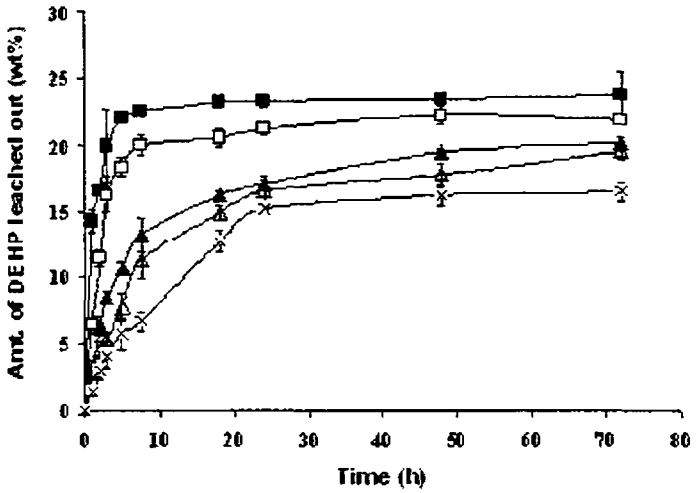


Figure.3. 12 Migration of DEHP from PVC/XNBR systems containing different XNBR contents. ■ Control PVC, □ 7.5phr XNBR, ▲15phr XNBR, Δ 20phr XNBR and X 25phr XNBR.

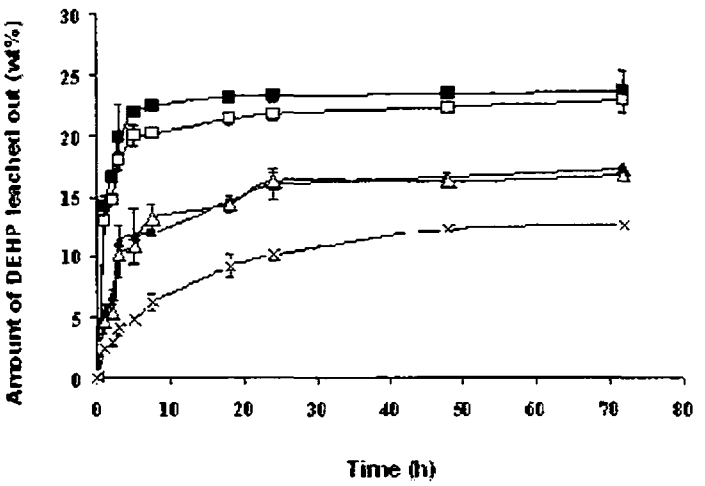


Figure.3.13 Migration of DEHP from PVC/ENR systems containing different ENR contents. ■ Control PVC, □ 7.5phr ENR, ▲ 15phr ENR, Δ 20phr ENR and X 25phr ENR.

3.3.3.2 Effect of Type of Elastomer

The comparison of the amount of DEHP leached out into the medium from the three systems in 72 hours was carried out and is as given in Figure 3.14. The effect of the elastomer content and the type of the elastomers can also be seen in the figuve. 80/20 and 75/25phr blends of PVC /NBR show less DEHP leaching compared to the corresponding PVC/XNBR and PVC/ENR blends. A delayed and reduced leaching of DEHP observed in the case of PVC/NBR samples further shows the superiority of NBR in resisting leaching compared to that of ENR and XNBR.

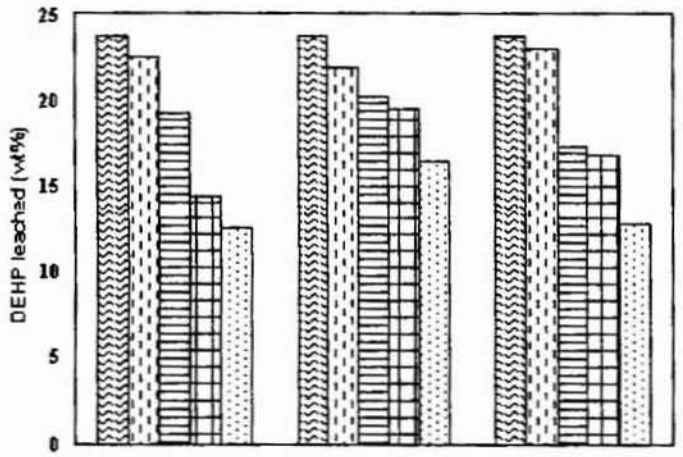
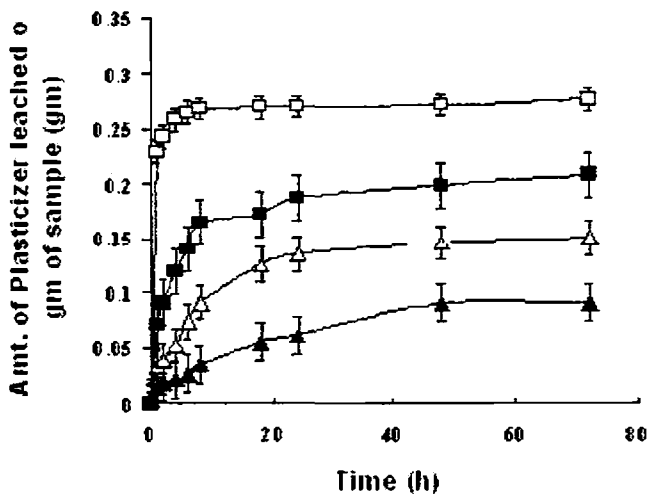


Figure.3. 14 Amount of DEHP leached out into petroleum ether at 30oC in 72 h from PVC/elastomer systems containing 0, 7.5, 15, 20 and 25 phr elastomer content

Of the three elastomers (NBR, ENR and XNBR) tried as partial replacement for DEHP in plasticized PVC, NBR was found to provide the most stable behavior with respect to mechanical behaviour as well as resistance to DEHP leaching.

3.3.3.3 Effect of Temperature

Various analytic methods have been reported for evaluating DEHP leaching from PVC articles and have indicated that DEHP leaching phenomenon is strongly influenced by the solvent used for extraction (25-27). Here, n-hexane has been selected as a leaching solvent to study the leaching trend of DEHP from control PVC and the NBR modified systems having different NBR and DEHP contents. Figures 3.15-3.17 show the extent of DEHP leaching from control and the NBR modified samples at 10, 25, and 40oC. The cumulative amounts of DEHP leached out per gram sample with leaching time are illustrated in these figures.



Figuve.3. 15 Leaching of DEHP at 10oC with time.

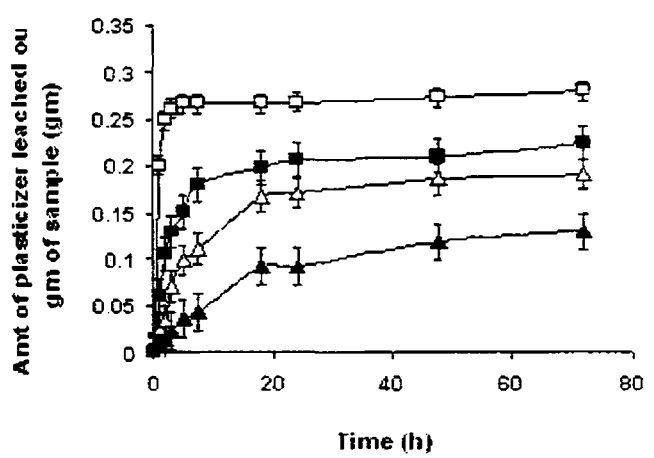


Figure.3. 16 Leaching of DEHP at 25oC with time

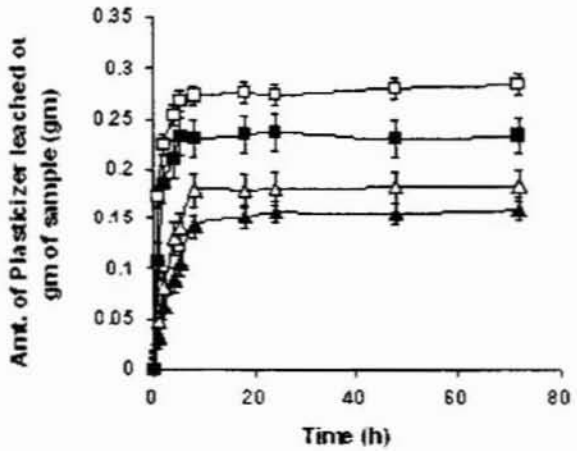
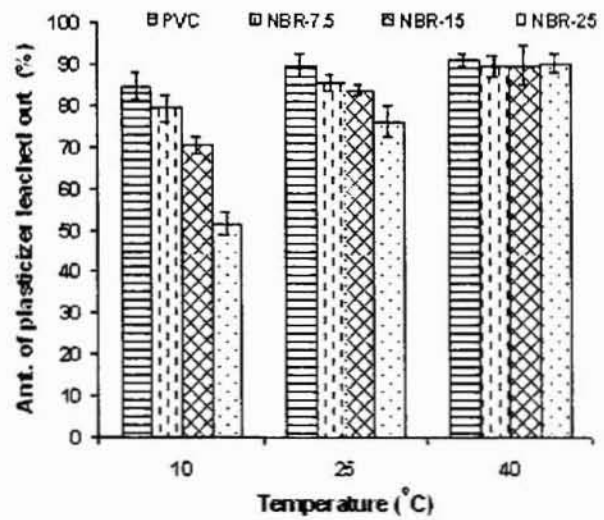


Figure.3. 17 Leaching of DEHP at 40oC with time

The results indicate that the leaching of DEHP into the medium from control sample is very fast and it reaches maximum level within 8 hours irrespective of temperature variation. But in the case of NBR modified samples the DEHP leaching at 40oC was completed in 18 h whereas at 10oC the leaching reached to the maximum level only in 24-48 h. It was further observed that the amount of DEHP leached out from NBR modified samples was strongly influenced by the temperature as well as the content of NBR. It may be seen from the graphs that the amount of DEHP leached out is directly proportional to the temperature and inversely proportional to the NBR content. It has been reported that nitrile rubber has got an affinity towards conventional liquid plasticizers(28). So the slow down in DEHP leaching pattern in NBR modified samples could be due to the affinity of the incorporated NBR towards DEHP.



Figuve.3. 18 Temperature dependence of DEHP leaching from Control and NBR modified samples in 72 hours.

Figure 3.18 shows the comparison of the percentage leaching of DEHP at different temperatures at the end of 72 h. It can be observed that the maximum retardation in leaching obtained by substituting DEHP by polymeric plasticizers is at lower temperatures.

The leaching, transferring and diffusion phenomenon of relatively small molecules through flexible polymers may be described by Fick's law applied to one dimension and may be expressed by the equation

$$\frac{M}{M_{\infty}} = 2\left[\frac{Dt}{\pi l^2}\right]^{\frac{1}{2}}$$

where, M_t and M_∞ are the measured quantities of DEHP migrated at time t and time infinity (72 h in the present study), D the diffusion coefficient unrelated to the DEHP concentration and l the thickness of specimen.

Plots of M_t / M_∞ versus $t_{1/2}$ for various systems at 10, 25 and 40°C are depicted in the Figures 3.19-3.21 respectively.

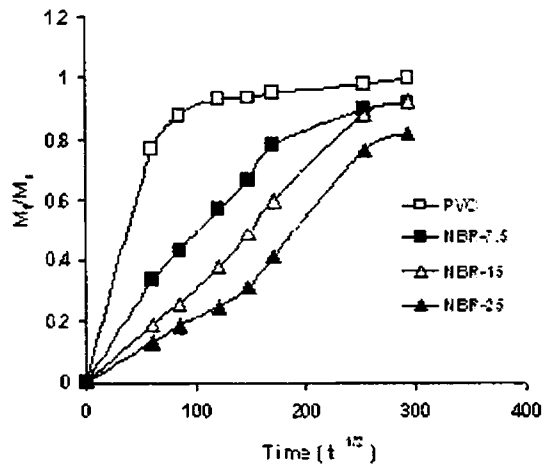


Figure.3.19 Plot of Mt /M∞ versus t1/2 for Control and NBR modified samples at 10oC.

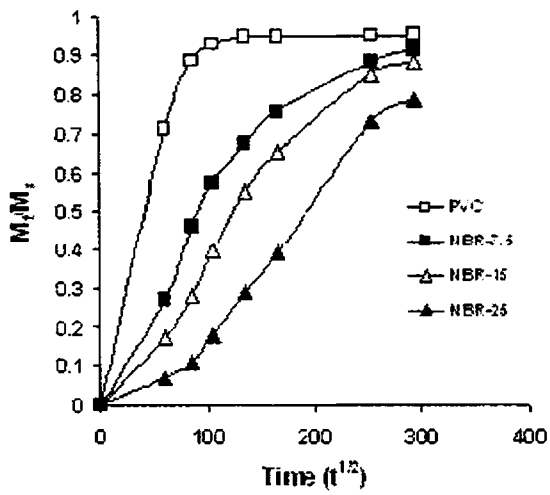


Figure.3.20 Plot of Mt /M∞ versus t1/2 for Control and NBR modified samples at 25 oC

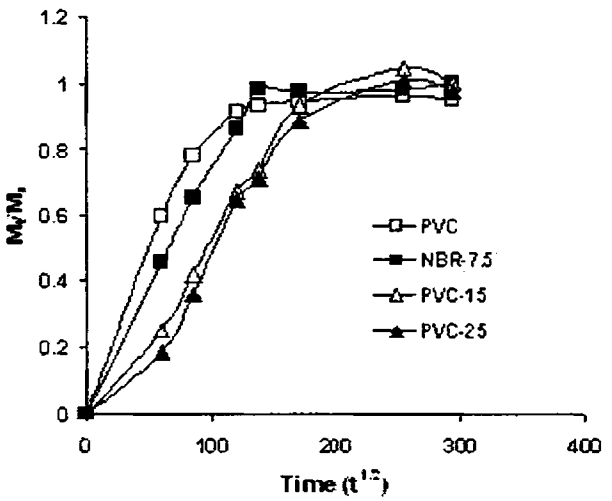


Figure.3. 21 Plot of Mt /M∞ versus t1/2 for Control and NBR modified samples at 40oC

As illustrated, M_t steeply increases in the initial period and then gradually reaches equilibrium. Furthermore, the time at which M_t reaches the plateau, decreased with increasing temperature. It suggests that DEHP leaching takes place very intensively at early stage in high temperature. According to eq. 4, a plot of M_t/M_∞ versus $t_{1/2}$ should yield a straight line of slope $2(Dt/\pi l^2)^{1/2}$. So, from the slopes, diffusion coefficient of each system was calculated and expressed in the Table 3.1. The high and low D values correspond to the large and small amounts of DEHP leaching respectively. The results indicate that except at 40oC, the diffusion coefficient decreases with increase of NBR content in the system. In other words, the increase of NBR content decreases the DEHP leaching rate. But at 40^oC the rate of DEHP leaching for all modified samples is more compared to the control PVC. Since the coefficient of thermal expansion of PVC (6.6-7.3x10-5) and that of NBR (19.6x10-5) are different, at higher temperatures polymeric chains of the individual components in the blends may undergo differential expansion, which can lead to the generation of small cavities that facilitate faster migration of the liquid plasticizer into the medium. Even though the rate of DEHP leaching is more at higher temperatures, the total availability of DEHP per gram of the sample is less compared to control.

Table 3.1 Diffusion Coefficient of Control and NBR modified PVC

Sample	Diffusion Coefficient		
	10°C	25°C	40°C
PVC	14.2	17.2	17.1
NBR-7.5	12.1	14.0	22.8
NBR-15	7.4	10.7	19.8
NBR-25	5.3	6.3	28.0

3.3.4 Density Measurements

Density data for NBR modified PVC samples given in Table 3.2 indicated only marginal variation from that of the control. The statistical evaluation of the data does not shows any significant variation in the densities of NBR modified samples except the sample containing 25phr NBR content.

Table 3.2 Density measurements data for Control and modified samples

Sample	*Density (gm/cc)*
Control PVC	1.21 ± 0.0023
NBR-7.5	1.209 ± 0.0035
NBR-15	1.207 ± 0.0069
NBR-25	1.215 ± .0015

3.3.5 Hardness Studies

Hardness is another important property of flexible PVC that has to be carefully monitored for biomedical applications. Softness or flexibility of PVC film and tubing is normally expressed in terms of durameter and can be tailored or fine-tuned to a specified number by adjusting the loading of plasticizer. Table 3.3 shows the shore-A hardness of control and modified samples. An increase in hardness values are seen with an increase in NBR content in the NBR modified PVC samples. But the values are within the tolerance of the medical bag applications.

Table 3.3 Hardness of control and modified samples

Sample	*Shore A hardness*
Control PVC	83
NBR-7.5	85
NBR-15	85
NBR-25	89

3.3.6 Gas Permeability Studies

Another important characteristic of blood component storage containers is gas-permeability. The stability and survival of the blood components for prolonged periods depends on the storage conditions as well as the O_2 and CO_2 permeability of the materials. During storage, blood components, for example, the platelets convert glucose, present in the anticoagulant to lactic acid and CO_2 (29). The CO_2 thus produced lowers the pH, which in turn adversely affects the stability of the blood components. However, the presence of O_2 suppresses the conversion of glucose to lactic acid and CO_2. So, for the extended life of the living blood components and longer storage times, the exchange of oxygen and carbon dioxide ie. the gas permeability is essential for the material used for the living cells of the blood component storage application purposes.

3.3.6.1 Effect of Elastomer Content

Gas permeability values of the control and modified PVC samples containing different NBR contents are given in the Table 3.4. A reduction in O_2 and CO_2 permeability was observed for all modified samples compared to that of control and the reduction being more prominent for CO_2. It was further noticed that the replacement of DEHP with NBR progressively reduces the gas permeability of the NBR modified samples. It has been indicated that the gas transmissibility of PVC formulation was dependent on the total amount of DEHP present and the lower transmissibility was obtained with lower DEHP content. Shang, et al. also in their patent on 'Plastic formulations for platelet storage containers and the like' indicated with regard to PVC plastic formulations that as the amount of plasticizer decreases, gas permeability generally decreases (30). Reduced gas permeability is not optimal for the storage of certain blood components, such as platelets.

Table 3. 4 O_2 and CO_2 permeability of control and NBR modified PVC at 10, 25 and 40°C

Material	Temperature	Permeability (cc/m².d)		Ratio
	(°C)	Oxygen	Carbondioxide	(CO_2/O_2)
PVC	14	671 ± 90	1934 ± 105	2.88
	25	1153 ± 182	4371 ± 255	3.79
	40	2250 ± 327	8226 ± 470	3.66
NBR-7.5	10	448 ± 6	976 ± 35	2.18
	25	1348 ± 136	3613 ± 179	2.68
	40	2385 ± 60	5681 ± 234	2.38
NBR-15	10	315 ± 17	536 ± 12	1.70
	25	1365 ± 25	2953 ± 263	2.16
	40	1901 ± 42	4242 ± 426	2.23
NBR-25	10	286 ± 14	413 ± 9	1.44
	25	1072 ± 83	2797 ± 103	2.61
	40	1810 ± 95	3710 ±196	2.05

3.3.6.1 Effect of Temperature

Plots of ln P versus 1/T for O_2 and CO_2 permeabilities of control and NBR modified PVC samples at 10, 25 and 40°C are shown in Figure 3.22 and 3.23 respectively. A linear relationship is obtained in the temperature range of 10-40°C. This indicates that the Arrhenius expression governs the temperature dependence of permeabilities of control and NBR modified PVC samples.

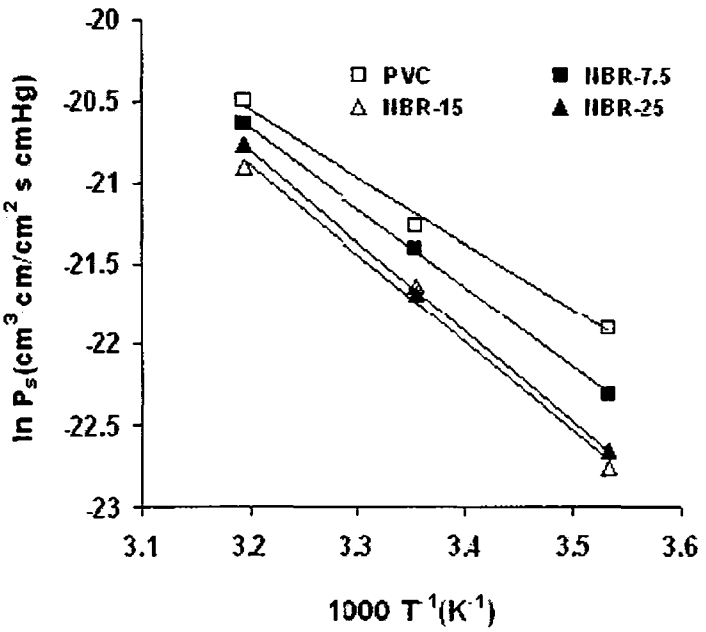
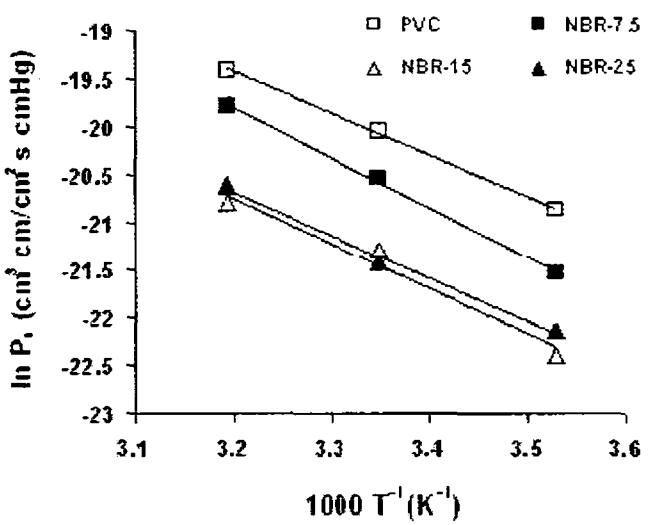


Figure.3.22 Temperature dependence of oxygen permeability of Control and NBR modified samples.



Figuve.3.23 Temperature dependence of carbon dioxide permeability of Control and NBR modified samples.

3.3.7 Water Permeability Studies

Specific water vapour transmission rate (Sp.WVTR) of control and NBR modified PVC samples at 25°C are shown in Figure 3.24. It is apparent from the figure that NBR influences the Sp.-WVTR of the modified samples, which indicates that the diffusion of water vapour through the sheets was increased with the increase of NBR content and reached to a maximum value for the system having 15phr NBR content. Further increase in NBR content was found to produce no appreciable variation in the water vapour transmission rate.

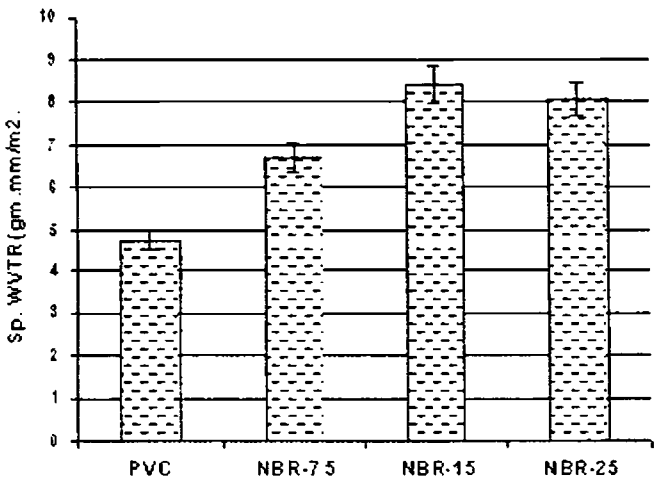
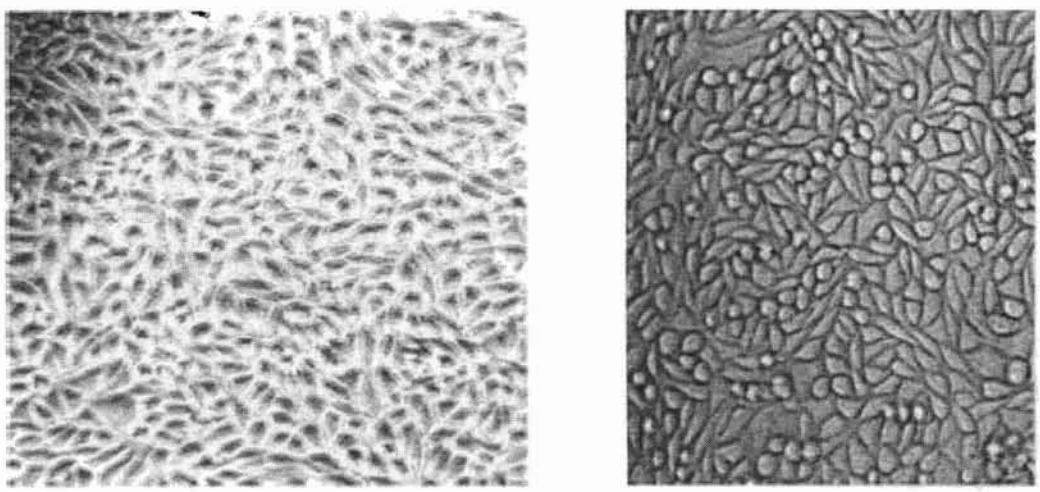


Figure.3.24 Specific water vapour transmissions rate of PVC and NBR modified PVC samples at 25°C.

3.3.8 In-Vitro Cell Culture Cytotoxicity Studies

To evaluate the possibility of the toxicity of NBR modified PVC samples, a preliminary cytotoxicity evaluation of the sample having high NBR content (25phr) was carried out using mouse fibroblast cells. Cytotoxicity test is a rapid, standardized, sensitive, and inexpensive means to determine whether a material contains significant quantities of biologically harmful extractables. Neither the sample nor its extracts induced any morphological changes to the cells confirming the non-toxic nature of the NBR modification. The morphology of the cells growing on the surface (scored as zero) is shown in Figure 3.24(a). It is clear from the figure that the typical spindle morphology of L929 was retained even after 24 h of contact with NBR modified PVC. Similar results obtained from the test performed on the extract of the sample indicate that there was no toxic material leached out of the sample Figure 3.24(b).



a b

Figure.3.25 L929 cells incubated with (a) NBR-25 (direct contact) and (b) extract from NBR-25 (test on extract) over 24 h.

3.4 Conclusions

Of the three elastomers (NBR, ENR, and XNBR) tried as partial replacement for DEHP in plasticized PVC, NBR is found to provide the most stable behavior with respect to mechanical behavior and resisitance to leaching

Reduction of DEHP leaching from PVC medical products minimizes the risk of DEHP contamination of the media, which comes in contact with it. Furthermore, reduced leaching of DEHP alleviates the problems of stiffening, mechanical property deterioration and transparency of PVC. The non-toxic nature of the material is revealed in the preliminary toxicity evaluation by in

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Chapter 4 METALLOCENE POLYOLEFIN: A POTENTIAL CANDIDATE FOR THE REPLACEMENT OF FLEXIBLE POLY (VINYL CHLORIDE) IN MEDICAL FIELD

4

METALLOCENE POLYOLEFIN: A POTENTIAL CANDIDATE FOR THE REPLACEMENT OF FLEXIBLE POLY (VINYL CHLORIDE) IN MEDICAL FIELD

4.1 Introduction

Due to the growing public concern about the environmental impacts of PVC (1,2) and the alleged toxicity of its plasticizer, DEHP (3,4), the search for an alternative material is on particularly for biomedical applications. Many materials like ethylene vinyl acetate (EVA) copolymer, thermoplastic polyurethanes (TPU), silicone, and polyolefins etc were tried as a replacement for flexible PVC in medical field (5-7). Even though the performance properties of thermoplastic polyurethane, silicones and ethylene-vinyl acetate copolymers are matching to those of flexible PVC along with added advantages of the absence of halogen plasticizer in it, these materials have other limitations. Silicone and TPU films are generally three to six times more expensive than PVC films on a weight basis, and may be over-designed for some applications. EVA films offer a 25% yield improvement due to their lower density compared with PVC, but EVA is also not widely accepted in place of pPVC due high material cost, reduced strength and tackiness.

Among the polymers investigated as alternative to PVC, the polyolefins are found to be more prominent. Polyolefins have been used for decades in the industry and are preferred for many applications because of their ease of processing by injection molding, blow moulding or extrusion; their durability, colorability, and cost-effectiveness. Recent progress in metallocene technology, has led to the development of cheaper metallocene-based polyolefin materials. Metallocene polyolefins (mPOs) have the potential to achieve much better performance than the conventional polyethylenes and polypropylenes. Because they have properties similar to many speciality polymers and engineering plastic, mPO have the potential to replace PVC and some expensive engineering plastics, particularly for medical products requiring high impact strength

and ductility at low temperatures (6). A comparative assessment of the performance properties of mPO with those of pPVC and EVA-18, the two common polymers used for flexible medical products are carried out in this chapter.

4.2 Experimental

The virgin polymers pPVC, mPO and EVA18 were melt mixed a torque Rheometer (Thermo Haake Rheocord 600) at 160°C using cam rotors with a rotor speed of 40rpm for 6 minutes. The torque values of mixing were monitored. TGA analysis of mPO was carried out between the temperature range form 23°C to 800°C at a heating rate of 10°C/min and DSC analysis of mPO was done as per ASTM E1356-98. The tensile properties of the samples were determined using dumb-bell shaped samples on a universal testing machine (Instron) at a crosshead speed of 100mm/min according to ASTM D638-03. The tear strengths of the blends were measured using MFI tester (Frank Devices, Italy) as per ASTM D1238-04c specification. The water contact angles of the samples were measured using a goniometer at 23 ± 2°C. Amount of the water absorbed by the samples were determined for a time period of 72 hours. Transparency of the samples was measured at 540nm using a UV-Visible spectrophotometer as per ASTM D1746-97. Water vapour transmission rates (WVTR) of the samples for 24 hours were measured according to ASTM E96-95. The oxygen and carbon dioxide gas permeability measurements were done in a manometric gas permeability tester at three different temperatures (10, 25 and 40^{o}C) as per ASTM D1434-98. The preliminary toxicity of the material was evaluated by conducting the cytotoxicity test using L929 mouse fibroblasts cell line as per ISO10993 test methods. The material mediated haemolysis was analyzed by doing the haemolysis test as per ASTM F 756-00. The blood clotting time was measured using fresh human blood according to Xianghuai et al.

4.3 Results and Discussions

4.3.1 Processability Evaluation of M-PO

Torque vs time curves or rheograms of mPO, PPVC and two grades of EVA are given in Figure 4.1. They represent the rheologcial characteristics of the samples during melting. The curve displays an initial increase, which is due to the loading of the material, subsequent decrease and final stabilization of the mixing torque. The stabilized torque value for mPO is lower than that of pPVC. While mixing at 160^{o}C, a stabilized level had been reached within 2 minutes and there was not much deviation from the stabilized level in the torque during the entire period of mixing for 6 minutes. This indicates no appreciable degradation of the materials during the mixing at 160^{o}C. The torque values indicate that the material is stable without appreciable degradation at this shear and temperature.

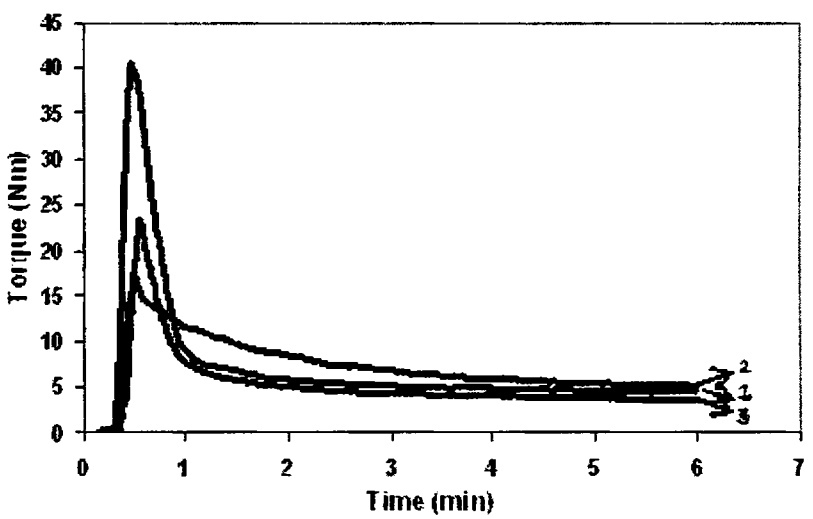

Figure 4. 1 Mixing torques of (1) mPO, (2) PVC, (3) EVA-1810

Apparent viscosities of samples are tabulated in the Table 4.1. The values indicate that the melt viscosity of mPO is lower than that of pPVC. This indicates that the processing of mPO is more economical than that of pPVC due to the utilization of less amount of mixing energy.

Table 4. 1 Melt viscosity of the samples

Sample	*Mixing Torque at 5minutes*	*Melt viscosity*
m-PO	4.4	1.05
PPVC	5.5	1.31
EVA-1810	3.6	0.86

The processability of a polymer is controlled by many factors like molecular weight, molecular weight distribution, melt viscosity, density and long chain branging which are all related to flow (8). It has been indicated that the narrow MWD of mPE makes them less sensitive to shear rates. The reduced shear thinning at the time of processing demands higher energy requirements for processing and the low level of shear-thinning results in processing difficulties, such as shark-skin in extrusion. These processing difficulties can be overcomed by the incorporation of short and long chain branching (9). Long chain branching tends to make the molecule more compact for equal molecular weights, and hence more shear thinning at higher shear rates. The torque and viscosity results obtained for mPO used in this study are more or less similar to those of flexible pPVC and EVA containing 18% vinyl acetate content. The comparable processibility of mPO with that of pPVC and EVA may be due to the alpha-olefin (octane) branches introduced by the manufacturer at the time of its production by using a special type constrained geometry metallocene catalyst (10,11).

4.3.2 Thermal Analysis

To find out the thermal degradation behaviour of mPO, a thermo gravimetric analysis of the sample was carried out in the temperature range from room temperature (23 ± 2°C) to 800°C.

It can be inferred from the Figure 4.2 that the thermal degradation of mPO takes places at the temperature above 400°C, which is much higher than its processing temperature. The DSC thermogram show in the Figure 4.3 indicates that the melt temperature of mPO and it can be processed above 107°C.

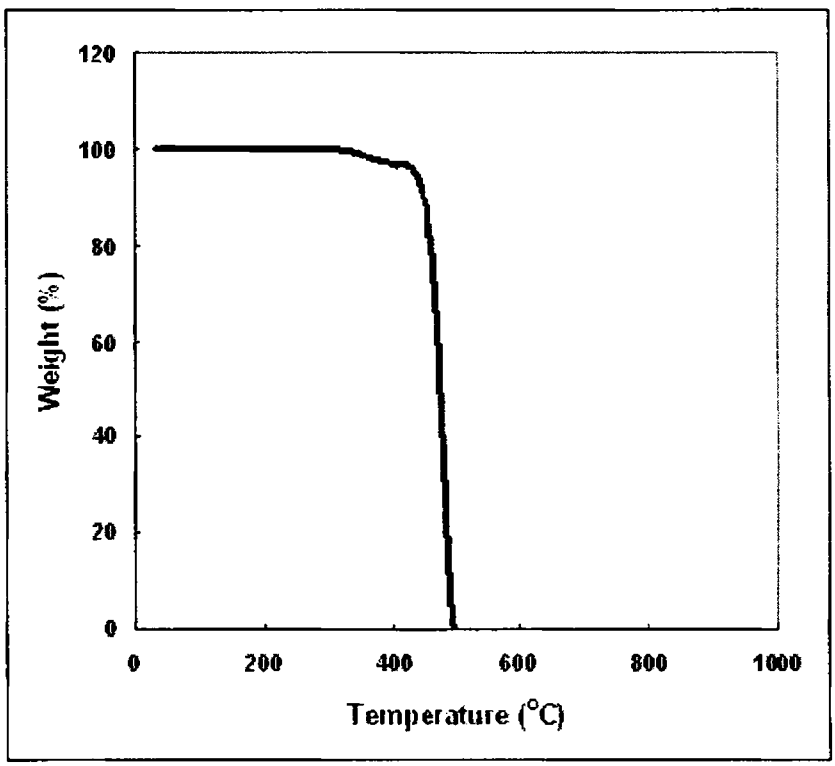

Figure 4.2 Thermal degradation profile of mPO

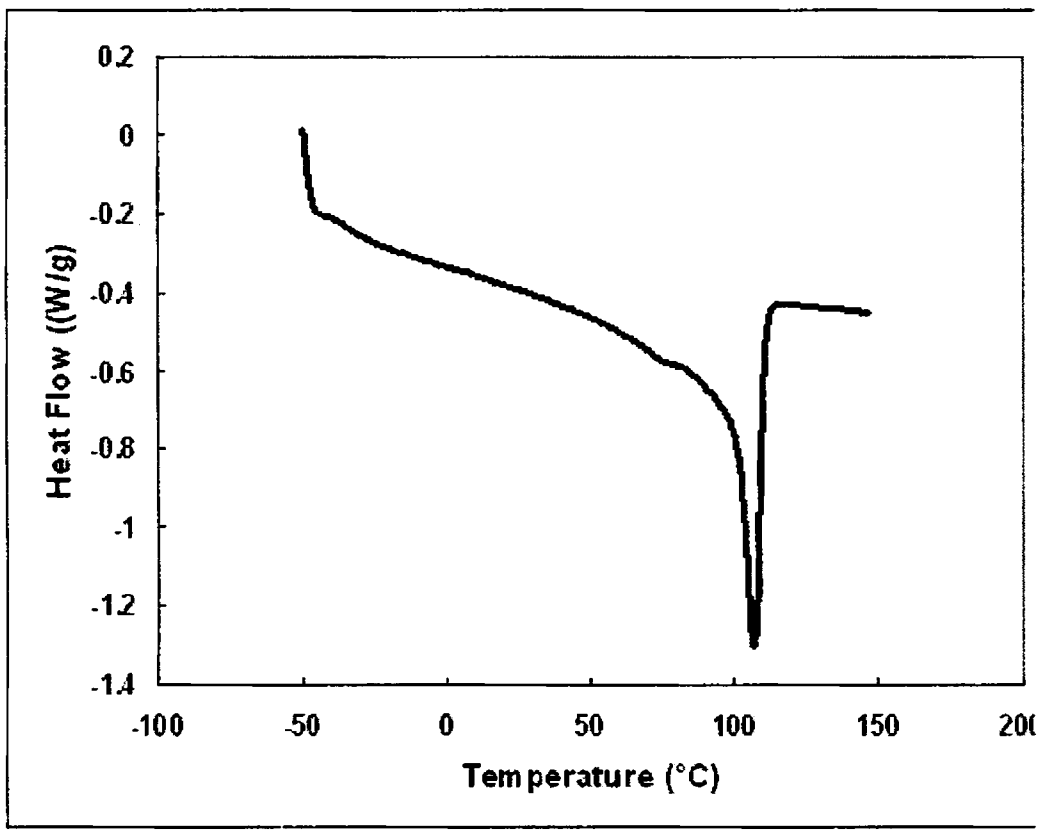

Figure 4.3 DSC thermogram of mPO

4.3.3.1 Stress – Strain Test

The tensile strengths and the percentage elongations at break of the samples are shown in figure 4.4 and figure 4.5 respectively. Both tensile strength and percentage elongation at break of mPO are higher than those of pPVC and EVA.

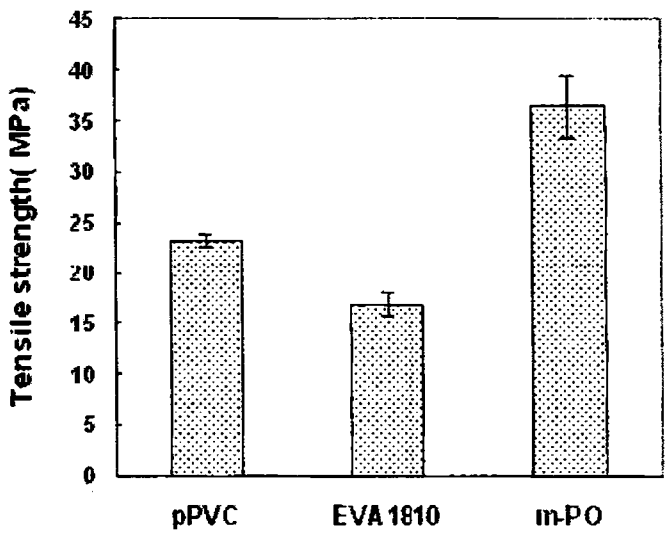

Figure 4. 4 Comparison of tensile strength of mPO with pPVC and EVA18

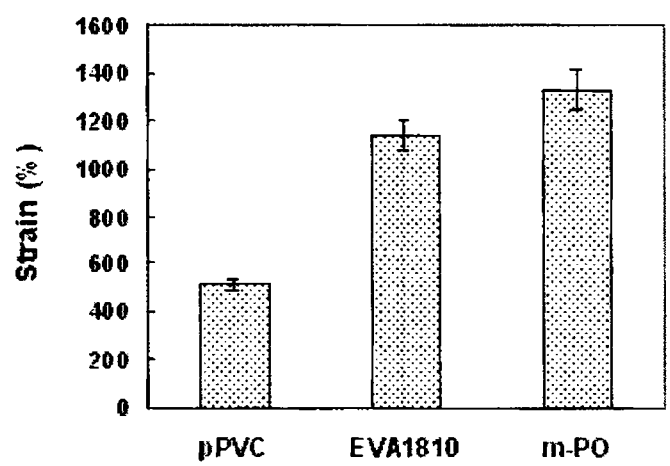

Figure 4. 5 Comparison of percentage Strain of mPO with PPVC and EVA18

Stress at different percentage Strain curves of pPVC with that of mPO and EVA18 are shown in figure 4.6. The nature of the curves indicating high modulus and impact strength for mPO

compared to pPVC and EVA18. Higher impact strength and modulus may be considered positive attribute of mPO over pPVC and EVA (12-13).

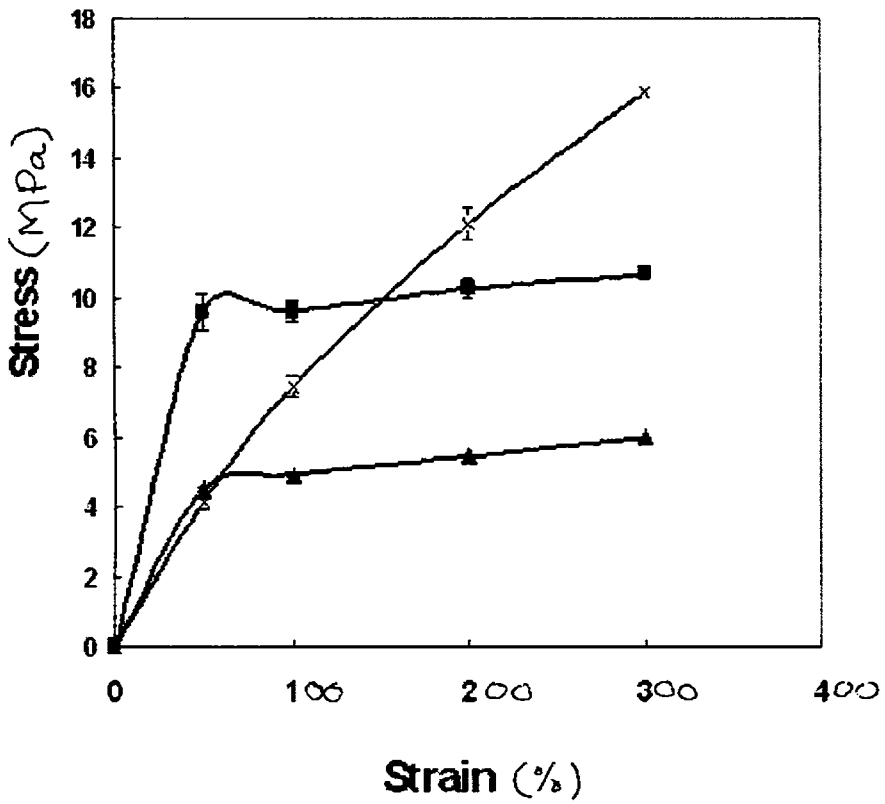

Figure 4. 6 Stress vs Strain curve X-PPVC, ▲- EVA1810, ■-mPO

4.3.3.2 Hardness Studies

Hardness is the measure of plastic deformation and is defined as the force per unit area of indentation or penetration and thus has the dimension of stress. Hardness values of the samples are given in Figure 4.7. The hardness of mPO is found to be higher than that of pPVC and EVA. It has been indicated that a material that can withstand high stresses and will undergo considerable plastic deformation (hard and tough material) is usually tougher than the one that has high capacity for deformation but can only withstand relatively low stress (soft and tough) (14). So, the hardness results further indicate that the impact resistance of mPO would be higher than those of pPVC and EVA.

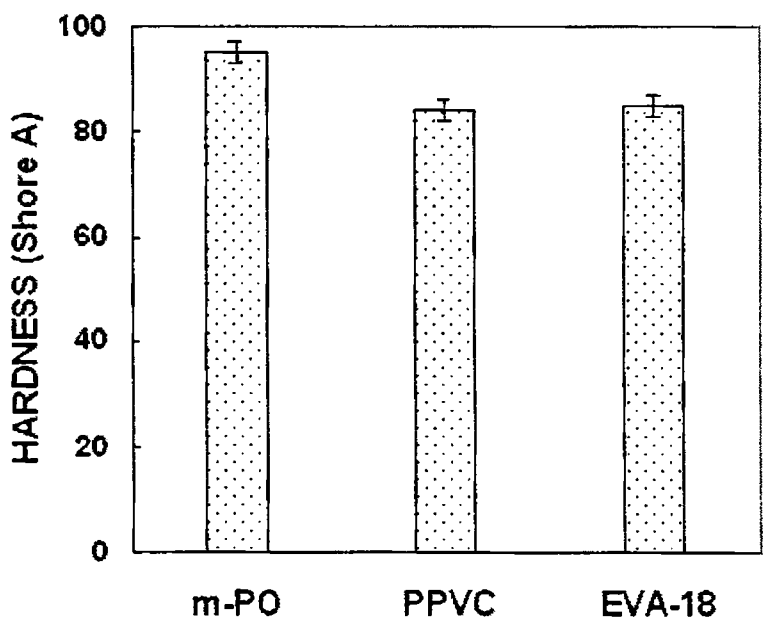

Figure 4. 7 Hardness of the samples

4.3.4 Transperancy

Transparency or clarity of the medical products such as blood or blood component –collection units and solution bags is an essential requirement (15). It enables the user to observe the unwanted happenings like coagulation of blood and blood components or growth of microorganisms in the fluid stored in. The percentage transmittances of the samples to a visible light at a wavelength of 540 nm are shown in the Figure 4.8. A higher transparency compared to pPVC was observed for mPO. High clarity has been reported for metallocene polyolefins films that make them quite suitable for many medical device and packaging applications (12,13).

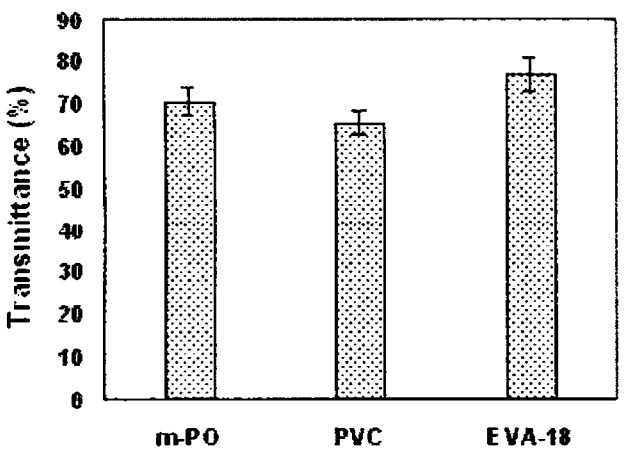

Figure 4.8 Percentage transmittance of the samples

4.3.5 Water Vapour Transmission Rate

The technique used to measure water vapor transmission rate (WVTR) was a modification of the wet cup method described by ASTM E 96-95. The mass of water loss from the dish was monitored as a function of time and the WVTR was calculated from the steady state region using the following equation. The WVTR is expressed as cc.mm/m^2.time.

WVTR = mass of water lost/time x area ----------(4.3)

Because the thickness of the sample varied, the WVTR is sometimes normalized to film thickness to obtain the specific water vapor transmission rate.

Specific WVTR = WVRT x thickness of the sample--------(4.4)

For a solid polymer, water vapor permeates the film by sorbing at the entering face, dissolving and rapidly establishing equilibrium, diffusing through the flim, and desorbing at the exit face. The mechanism of permeation involves both solution and diffusion (16).

Water vapour transmission rate of the material indented for medicated solutions as well as blood and blood component storage application purposes should be minimal. Loss of water from the medicated solution stored inside the plastic container alters the concentration of the medication,

which in turn changes the intended dose. In the case of blood and blood components loss of water adversely affect the composition and thereby the stability of the body fluids inside the container. Figure 4.9 shows the sp.WVTR of the samples at 23°C. Specific WVTR of the samples were calculated per unit area and unit thickness of the samples. The results show that the Sp.WVTR of mPO is lower than those of plasticized PVC and EVA18. And it is in the order of mPO<EVA-18<pPVC. Better water-vapor barrier property of polyolefin has been indicated by Robert Kelch in his study on HF- weldable polyolefin films (17). The same trend is observed in the WVTR for mPO, pPVC and EVA.

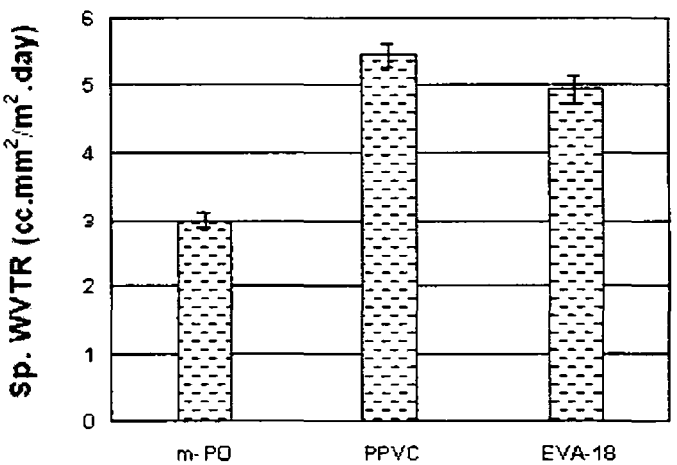

Figure 4. 9 Sp.Water vapor transmission rate of the samples at 23°C

The effect of temperature on water vapor transmission rate can be seen in the figure 4.10. The experiment was carried out at three different temperatures (23, 37 and 50°C) and the results indicated an increase of WVTR with an increase of temperature. Moreover, increased differences in the WVTR were observed between the samples at elevated temperatures. This observation is well in accordance with the following equations for sorption (S) and diffusion (D), the mechanisms by which the water vapor permeation takes place in film samples.

$S = So \exp(-Hs/RT)$ ---------- (4.5)

$D = Do \exp(-ED/RT)$ ----------(4.6)

Where, Hs is the apparent heat of solution and ED the activation energy for the diffusion process and the subscript zero refers to a standard state. As per the above equations it can be seen that the sorption and diffusion increase with increase of temperature, which in turn increase the water vapor transmission rate.

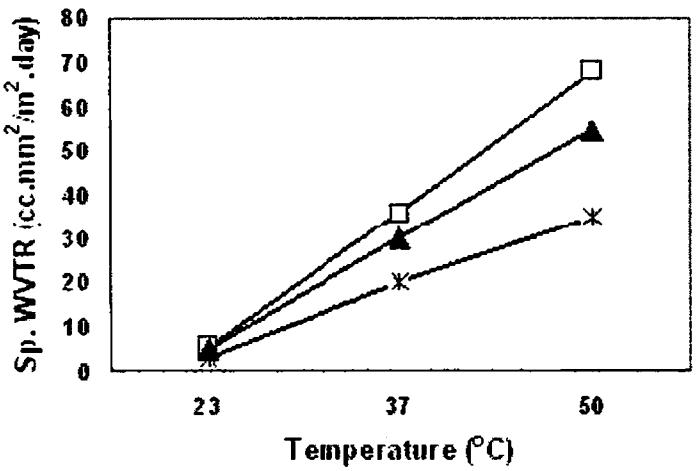

Figure.4.10 Sp.Water vapor transmission rate of the samples: Effect of Temperature ✳ m-PO, - PPVC and ▲ EVA18

4.3.6 Gas Permeability Studies

Gas permeability of the container used for blood or blood component storage applications is an important parameter. Gas permeability is essential so that the living cells of the blood component, such as red blood cells and platelets, can exchange oxygen and carbon dioxide. This allows for the extended viability of the living blood component and longer storage times. With regard to PVC plastic formulations, as the amount of plasticizer decreases, gas permeability generally decreases. Reduced gas permeability is not optimal for the storage of certain blood components, such as platelets.

Blood has been collected in bags containing buffered anticoagulants such as ACD (acid citrate-dextrose) or CPD (citrate-phosphate-dextrose). Platelet concentrates also contain glucose (dextrose) as a consequence of the process by which they are collected. During storage, the platelets convert glucose to lactic acid and carbon dioxide (CO_2), which lower the pH. Murphy and Gardner measured CO_2 and oxygen pressures in various PVC and polyethylene (PE) bags containing platelet concentrates and observed that the drop in pH was greater in thicker the walls of the bag (18). The pH of storage is critical. The pH falls during storage due to the production of lactic acid (anaerobic metabolism) and carbon dioxide (aerobic metabolism). Platelets undergo a disc-to-sphere transformation as the pH falls from its initial value of 7.0; they become swollen and irreversibly damaged at a pH less than 6.2. This pH change limits the duration of storage. A high pH (> 7.8) is also associated with loss of viability.

Since oxygen is known to suppress conversion of glucose to lactic acid, it was concluded that the efficiency of oxygen transport into and CO_2 transport from the bags was dependent upon the thickness of the bag walls. For a given platelet count, the pH drop of stored concentrates was

significantly less for thin walled containers. Concentrates with high platelet counts stored in standard PVC bags having considerably thicker walls had a pH of around 6.0 or lower after 3 days storage. Murphy and Gardner indicated an abrupt loss of in vivo viability of platelets if pH falls below 6.0 during storage (18). Due to the plasticizer leaching problems associated with PVC and the high susceptibility of conventional polyethylene towards rupturing during pressure steam sterilization and/or centrifugation, it would therefore be highly desirable to be able to store platelets in a plastic container having sufficient tensile strength to withstand pressure sterilization and high-speed configuration while at the same time having good carbon dioxide and oxygen permeability characteristics so as to prolong platelet survival. It would be a particular advantage if platelet survival could be prolonged beyond the usual three days survival period. Currently, blood banks must discard platelets after three days storage that makes it difficult and expensive to maintain supplies for emergency situations.

As the metallocene polyolefins possess most of the desired properties for packaging applications especially the high tensile strength to withstand the high-speed centrifugation, this material can be a suitable candidate for body fluid storage application purposes. Oxygen and carbon dioxide permeability of mPO were evaluated and compared with those of pPVC and EVA-18 as per the ASTM 1434-95 method. The results of oxygen and carbon dioxide permeabilities of mPO, pPVC and EVA, at three different temperatures (10, 25, 40°C) are as shown in figure 4.11 and Figure 4.12 respectively. It has been seen that the permeabilities of both gases for mPO at three temperatures are lower than those of pPVC and EVA. But in medical field, the material used for collection and storage of body fluids should have more CO_2 permeability. Otherwise the CO_2 produced by the platelets and other cells inside the container may dissolve in the fluid and cause the fluid pH to drop and which in turn decrease the pH of the fluid stored in and may damage the platelets and other cells of the fluid.

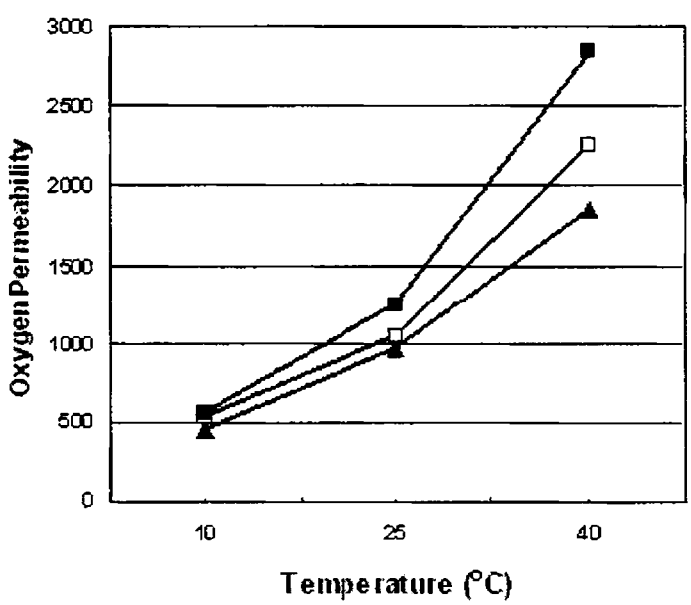

Figure 4. 11 Influence of temperature on the oxygen permeability pPVC ■ EVA and ▲ mPO

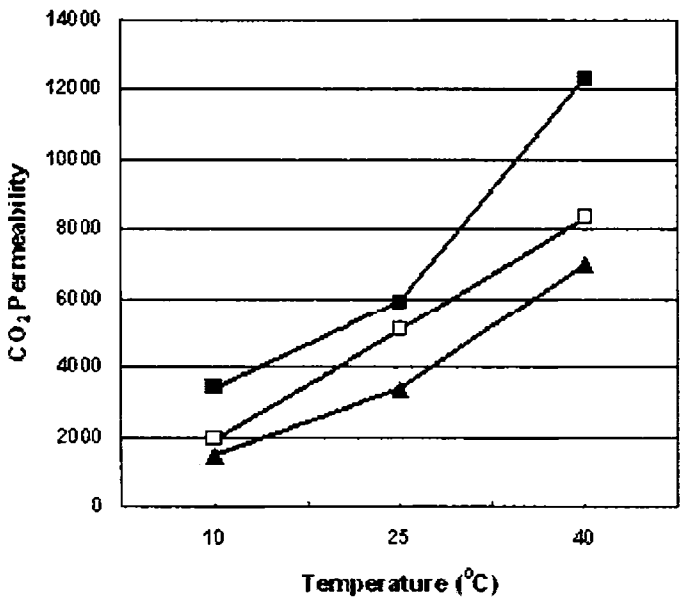

Figure 4. 12 Influence of temperature on the carbon dioxide permeability. pPVC ■ EVA and ▲ mPO

The results further showed that the permeabilities increased with the increase of temperature.

For collection or storage containers of body fluids, the ratio of carbon dioxide to oxygen permeabilities is very important. Higher value of this ratio is better for containers used for body fluid storage applications. The ratios of carbon dioxide to oxygen permeabilities of the samples at three different temperatures are given in the table-2. The low gas permeability and CO_2/O_2 ratio may increase concentration of the CO_2 inside the container, which in turn decrease the pH of the fluid inside from 7.4 to 6 or below.. Murphy and Gardner reported that an abrupt loss of in vivo viability of cells occurs if pH falls below 6.0 during storage (19). So the mPO in the virgin form cannot be considered as a potential candidate for blood/ blood component storage application purposes.

Table 4.2 Ratios of carbon dioxide to oxygen permeabilities of the samples at three different temp

Material	Ratio of CO_2/O_2		
	10°C	25°C	40°C
m-PO	2.75	3.45	3.23
pPVC	3.62	4.86	3.79
EVA-18	5.29	4.34	4.2.8

4.3.7 In Vitro Cell Culture Cytotoxicity

The response of mPO to a culture of L929 cells is shown in Figure 11(a & b). It is clear from the figures that the typical spindle morphology of L929 was retained even after 24 hours of direct contact with mPO (Figure 4.13 a) and the extract of the material (Figure 4.13 b). The results from the in vitro cell culture cytotoxicity revealed that the material mPO was non-cytotoxic to L929 cell line and no toxic leachables was leached out from the material to damage the test cells.

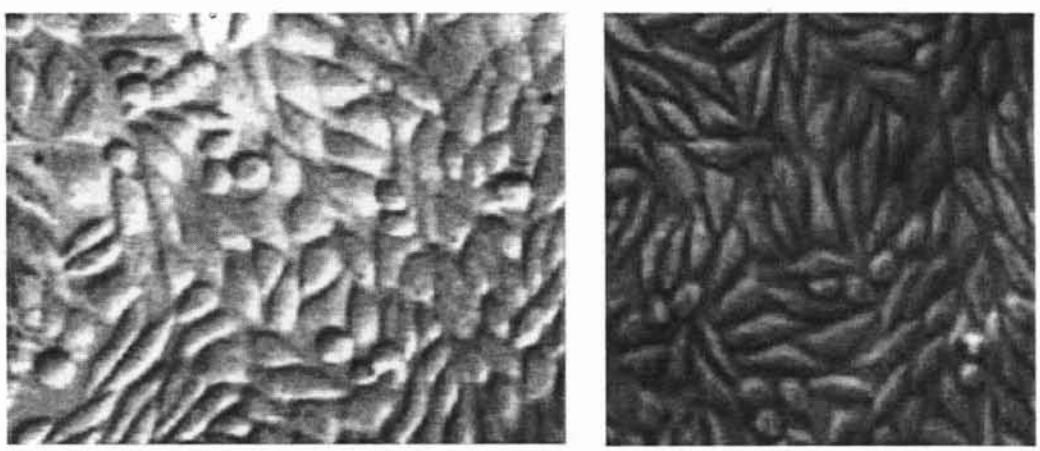

Figure 4. 13 L929 cells incubated with (a) m-PO and (b) extract from m-PO over 24h.

4.3.8 Whole Blood Clotting Time Test

When blood contacts with an artificial surfaces, a series of events is initiated: rapid adsorption of a layer of plasma proteins at the interface, platelet adhesion to the protein layer and activation of the coagulation system to form thrombin and fibrin and finally culminate in the clot formation (20,21). This fact can be used a test method for testing materials intended for blood contact applications. When blood comes in contact with a material and takes a long time to clot it means that material is better for blood contact application compared to material, which initiate the clot faster. In the whole blood clotting time test method a specific quantity (0.1mL) of blood was placed on the surface of the material. After a predetermined time the specimen was transferred into a beaker containing 50mL of water. The red blood cells that have not been trapped in the thrombus were haemolysed in the water and were measured at 540 nm in an uv-visible spectrophotometer. The variation of optical density of the solution with time was plotted as shown in the Figure 4.14. Conventionally, the time at which the optical density decreases to 0.1 is regarded as the clotting time. So in the test results mPO initiates the clotting cascade fastern (12minutes) than that of pPVC (19 minutes).

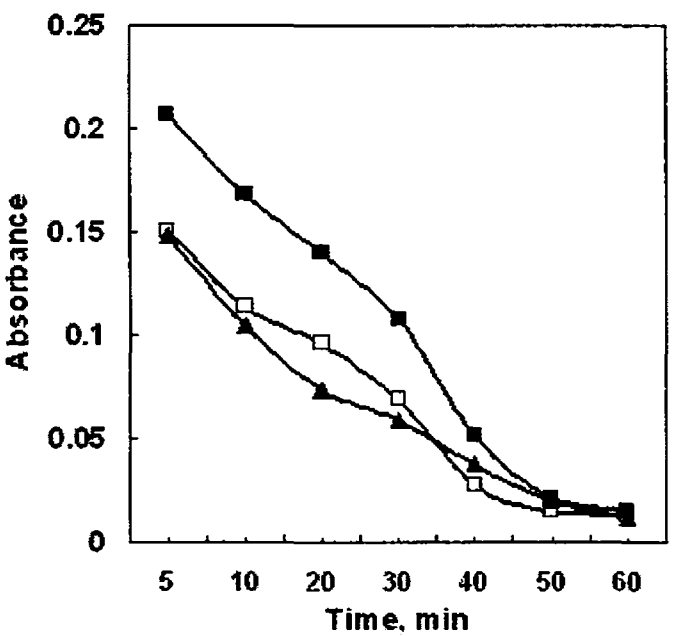

Figure 4. 14 Clotting time of ▲-mPO, □-ppVC, ■-EVA

4.3.9 In Vitro Haemolysis Test

Since hemolysis is a measure of the destruction of red blood cells, it is mandatory to conduct an in vitro haemolysis test for the material indented for blood storage applications. ASTM F 756-00, a standardized ASTM hemolysis test method, is available for determining the hemolytic potential of a device or material. This in vitro test involves a quantitative measurement of hemoglobin released from the red blood cells due to the lysis of cell membrane and thereby indicating hemolytic activity of the material exposed to the cells. Such testing is frequently performed using rabbit blood. These test were carried out in static as well as dynamic conditions. To evaluate the material mediated haemolysis (direct contact) and the haemolysis due to the leachable from the material (test on extract) two test methods were conducted. The results of haemolysis tests for static and dynamic conditions are as shown in the Figure 4.15 and Figure 4.16 respectively. According to ASTM F 756-00, the results of haemolysis test can be graded as indicated in the Table 4.3.

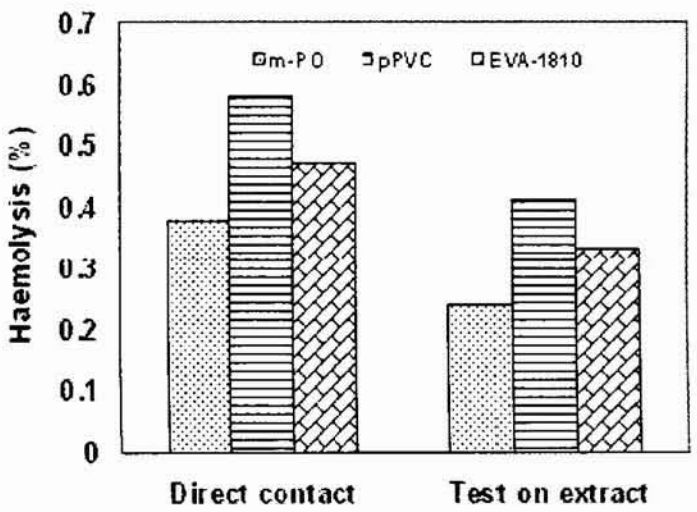

Figure 4.15 Percentage haemolysis for the samples at static condition

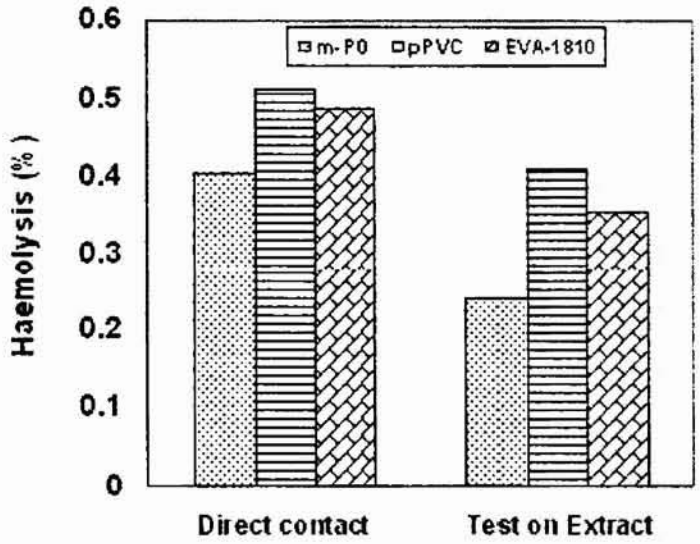

Figure 4.16 Percentage haemolysis for the samples at dynamic condition

Table 4.3 Haemolytic Grade with respect to haemolytic index

Haemolytic Index	*Haemolytic grade*
0-2	Non-Hemolytic
2-10	Slightly Haemolytic
10-20	Moderately Haemolytic
20-40	Markedly Haemolytic
Above 40	Severely Haemolytic

The haemolytic index for the material to be used for blood contact applications should be below 2%. So, results for all the samples are with in the limit of ASTM standard and the material mPO can be proposed for blood contact applications. In both static and dynamic conditions mPO showed less haemolytic index compared to pPVC and EVA1810. This indicates that the mPO is a better and a promising candidate for the replacement of pPVC especially in the blood contact applications field.

4.4 Conclusions

In this investigation, processing parameters like the mixing torque and melt viscosity, mechanical properties, gas and water permeability and some biological tests like cytotoxicity, whole blood cloting time, percentage haemolysis etc. of metallocene based polyethylene plastomer were evaluated and compared to those of pPVC and EVA. The processibility of the plastomer is found to be comparable to those of plasticized PVC and EVA. Mechanical studies indicate that mPO has better strength, elongation and toughness than pPVC and EVA. Carbon dioxide and oxygen permeabilities of mPO are lower than those of plasticized PVC and EVA. The material mPO is found to be noncyto toxic and less haemolytic whereas it initiates clotting faster than pPVC and EVA18. Even though the mechanical property advantage of mPO over pPVC and EVA is superior, the low CO_2 and O_2 permeability of mPO gases may restrict its use as container material for blood/platelet storage applications. As permeability is an important parameter of material used for blood/platelet storage purposes, mPO may have to be modified to meet the requirement.

4.5 References

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Chapter 5 MODIFICATION OF METALLOCENE POLYOLEFIN

5

MODIFICATION OF METALLOCENE POLYOLEFIN

5.1 Introduction

The advent of polyolefins made by metallocene catalysts offers new opportunities in the area of product development for the plastic industry. However, for specific applications or product's special requirements, a fine turning like structural modification of the polymer or blending of other additives is required. For example, In order to enhance the processability and improve other specific properties it is the common practice to use blends of polymers (1-5). In the previous chapter it has been seen that some of the key attributes of mPO are matching to the required properties of the material to be used for blood/blood component storage purposes. However the low gas permeability and faster material mediated blood clot formation while interacting with blood hinders its applicability for the storage of such fluids. So a modification on mPO is needed to adapt it to the required properties of the product.

The property evaluation studies of mPO, pPVC and EVA copolymer carried out in the previous chapter shows a high gas permeability and very good blood compatibility for EVA copolymer compared to pPVC and mPO. EVA copolymer has been used for the modification of metallocene polyolefins to improve it processability and heat seal properties (6-9). EVA may be good modifier for mPO for improving its gas permeability and slow down the blood clot formation. Modification of mPO with EVA in this perspective is described in this chapter.

5.2 Experimental

Metallocene polyolefin was modified by mixing with of two grades EVA having the vinyl acetate content 12 and 18%. The mPO/EVA blends containing 5, 15, 25, and 50wt% of EVA were prepared by mixing in a torque Rheometer (Thermo Haake Rheocord 600) at 160°C using cam rotors with a rotor speed of 40rpm for 6 minutes. The torque values of mixing were monitored. Melt flow index of the blends were measured according to ASTM D1238-04c. TGA analysis of the samples were carried out between the temperature range form 23°C to 800°C at a heating rate of 10°C/min and DSC analysis were done as per ASTM E1356-98. The tensile properties of the samples were determined using dumb-bell shaped samples on a universal testing machine (Instron) at a crosshead speed of 100mm/min according to ASTM D638-03. The tear strengths of the blends were measured using a universal testing machine at a crosshead speed of 500mm/min as per the ASTM designation D624-98. The melt flow indexes of the samples were measured suing MFI tester (Frank Devices, Italy) as per ASTM D1238-04c specification. The water contact angles of the samples were measured using a goniometer at 23 ± 2°C. Amount of the water absorbed by the samples were determined for a time period of 72 hours. Transparency of the samples were at 540 nm using a UV-Visible spectrophotometer as per ASTM D1746-97. Sp. water vapour transmission rates (Sp.WVTR) of the samples for 24 h were measured according to ASTM E96-95. The oxygen and carbon dioxide gas permeability measurements were done in a manometric gas permeability tester at three different temperatures (10, 25 and 40°C) as per ASTM D1434-98. The morphology of cryogenically fractured and etched surfaces of the samples were examined in scanning electron microscope. The pH of the extract of the samples before and after gamma radiation using Cyber scan 510 pH meter at 23 ± 2°C.

5.3 Results and discussion

It has been reported that the miscibility of the EVA resin with LDPE or LLDPE increased as the vinyl acetate (VA) content decreased (10). Moreover, Huang in his study on the blends of EVA and mPE reported that miscible blends could be obtained if only the VA content was around 18% (11). Hence we selected the EVA copolymers having 12 and 18%VA content for modification of mPO.

5.3.1 Mixing Torque Studies

The variation of the mixing torques of mPO, EVA12 and EVA18 with time are shown in Figure 5.1. A mixing time of 6 minutes was fixed since the torque stabilized to a constant value during this time in all cases. The temperature of the mixing chamber was fixed as 160°C. The stabilization of the torque may be related to the attainment of a stable structure after good level of mixing. The torque curves show that all the samples melt with in 2 minutes and reached to a stabilized level. The stability of the torque curves of all the samples after melting indicates that there was no degradation of the samples during the entire mixing period of 6 minutes. The mixing torque of EVA12 is marginally lower than that of mPO, whereas a marked difference in the torque values can be seen between mPO and EVA18. Usually, for metallocene polyolefins a high torque value is expected due to their narrow molecular weight distribution (12). But a comparable torque value of mPO and EVA12 may be mainly due to the more or less equal average molecular weight of mPO. However, the narrow molecular weight distribution of mPO can be observed its sharp initial melting peek. In the case of EVA12 and EVA18, fairly broad molecular weight may be smoothen out the melting whereas such an effect is absent in the case of mPO.

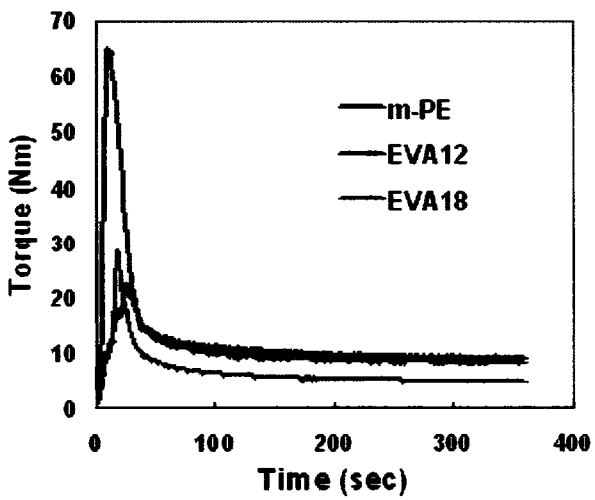


Figure 5.1 Variation of mixing torque with mixing time for virgin polymers

5.3.1.1 Effect of EVA content in the Blend

The variation of mixing torque with mixing time of the different composition of the blends of mPO and EVA12 is give in Figure 5.2. The stabilized mixing torque of virgin as well as the blended samples shows more or less similar values. However, the initial melting peak shows a distinct pattern. While the melting peak is maximum for mPO due to its low molecular weight distribution, it progressively come down with increase in EVA12 content.

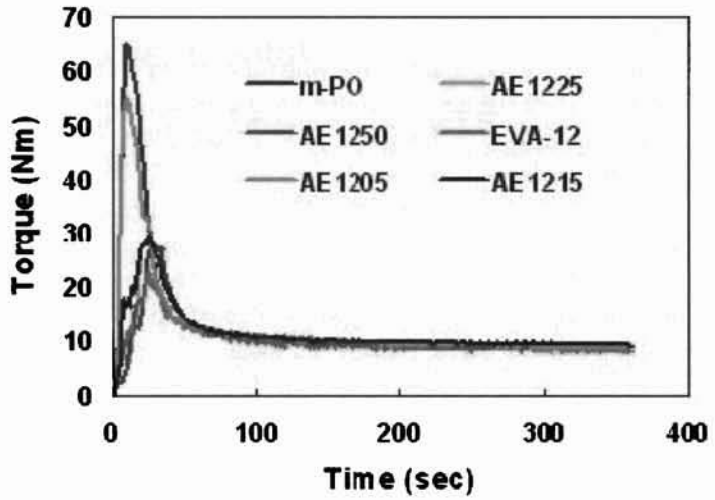


Figure 5.2 Variation of mixing torque of EVA12 blends with mixing time

The effect of EVA12 content on the stabilized mixing torque is shown in Figure 5.3. The torques of the blends at the stabilized level (6 minutes) are plotted against the percentage of EVA12 content in the blend. As the stabilized mixing torques of the virgin polymers are more or less equal, there is no much variation in the stabilized mixing torque values of the blends having different amount of EVA-12.

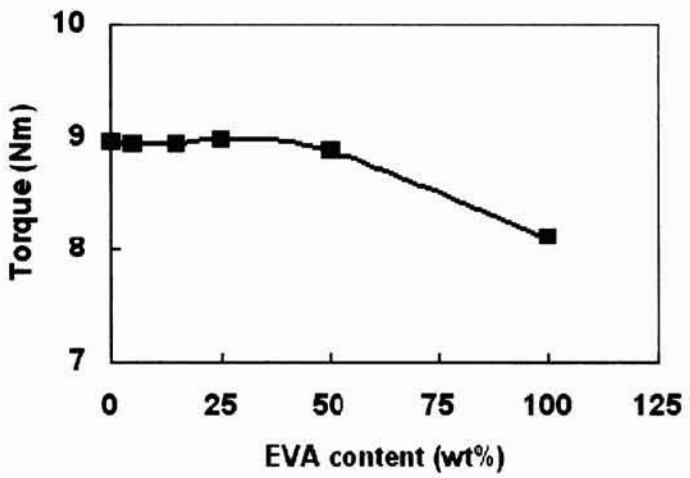


Figure 5.3 Effect of EVA content on the mixing torque of m-PE/ EVA12 blend with varying EVA12 contents.

The variation of mixing torque with mixing time of the various blend compositions of mPO and EVA18 are shown in Figure 5.4. The mixing torque pattern is similar to that of mPO/EVA12 blends. But a marked variation in the mixing torques of the blends after stabilization can clearly be seen in the figure.

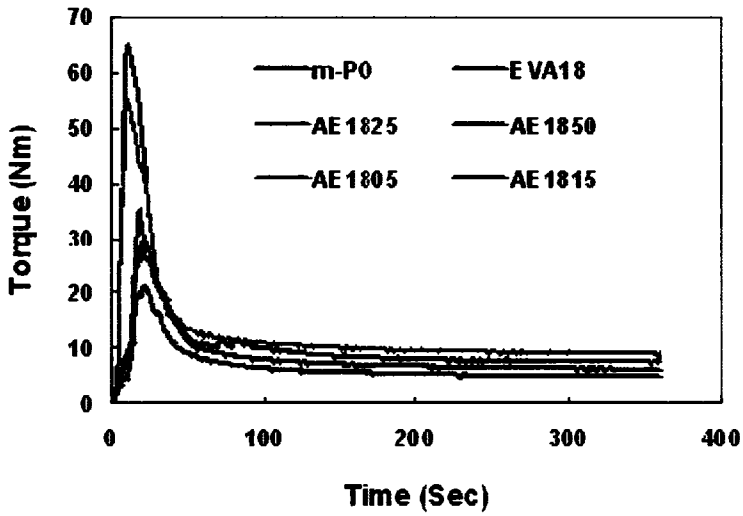


Figure 5.4 Variation of mixing torque with mixing time

To see the effect of EVA18 content in the mPO/EVA18 blend on the mixing torque, the stabilized torque values of the respective blends are plotted against the percentage EVA18 content in Figure 5.5. There is a progressive decrease in the torque values with of EVA18 content in the blend obviously due to the lower stabilized torque in the case of EVA18.

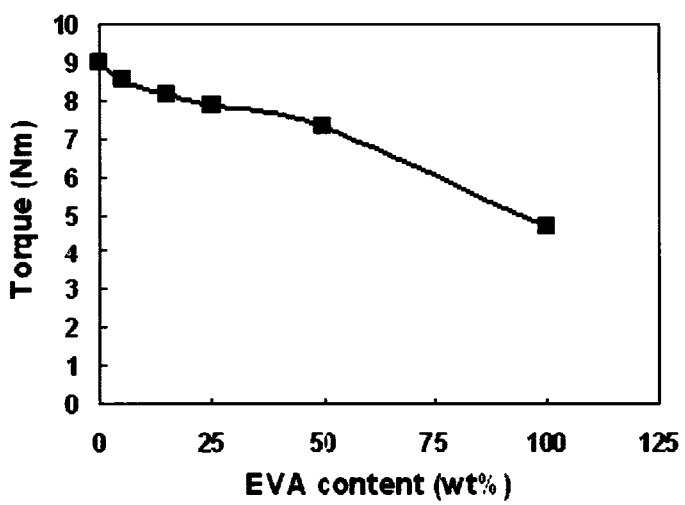


Figure 5.5 Effect of EVA content on the mixing torque of m-PE/ EVA18 blend with varying EVA18 contents.

5.3.1.2 Effect of Vinyl Acetate Content

Figure 5.6 shows a comparison of mixing torques of mPO/EVA12 and mPO/EVA18 blends. It can be noted that mPO/EVA12 blend show a higher torque when compared with the mPO/-EVA18 blend. This indicates that more energy is required to melt mix the blend mPO /EVA12 than that of mPO/EVA18. The Figure 5.6 further shows the comparison of mixing torque of the blends mPO/EVA12 and mPO/EVA18 with that of pPVC. All the blends of mPO with EVA12 as well as EVA18 show higher mixing torques than that of pPVC.

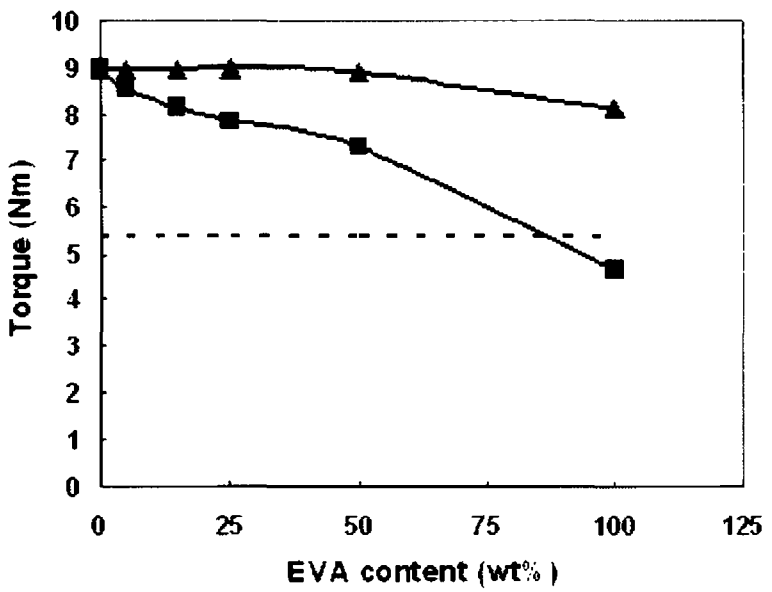


Figure 5.6 Effect of vinyl acetate content on the mixing torque of ▲- m-PE/EVA12, ■- m-PE/EVA18 blend and ---- pPVC_

5.3.2 Apparent Viscosity studies

5.3.2.1 Effect of EVA and Vinyl Acetate content in the blend

The effect of percentage EVA content in the two blend compositions on the melt viscosities at the time of mixing at a constant speed of 40 rpm and temperature 160°C is given in Figure 5.8. The melt viscosity of the blend mPO/EVA12 has no effect on the EVA content, whereas a decrease in the melt viscosity can be seen with the increase EVA content in the case of mPO/-EVA18 blend. The effect of vinyl acetate (VA12 and VA18) content in the blend on the melt viscosity is also seen in the picture. It indicates that as the vinyl acetate content increases the melt viscosity tends to decrease. On comparison of the melt viscosities of the blend with that of pPVC, it can be seen that all the blend compositions have higher melt viscosities. This indicates that the processability of the blends are slightly difficult than that of pPVC.

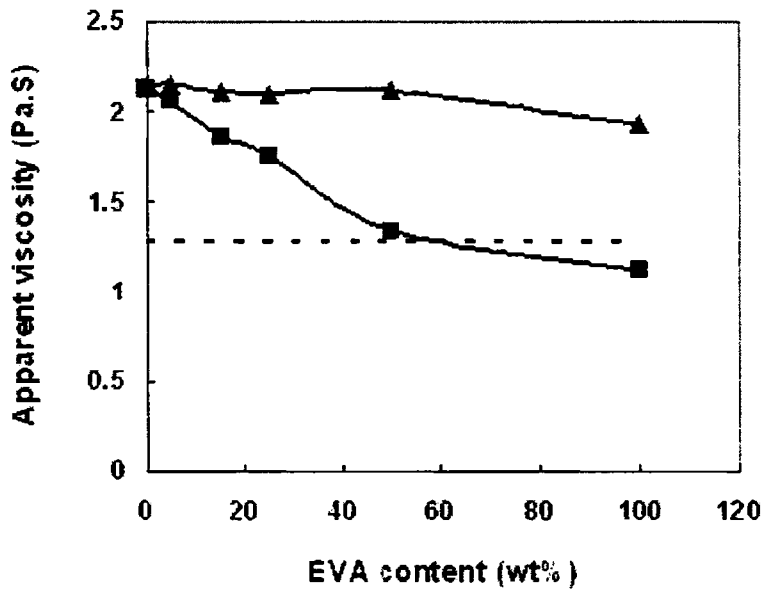


Figure 5.8 Apparent melt viscosity at mixing vs percentage EVA content ▲- m-PE/ EVA12■- m-PE/ EVA18 blend and ----- pPVC

5.3.2.2 Shear Thinning Effect

The effect of shear rate on the apparent viscosity of the virgin polymers and blends were investigated at 160°C over the of speed range 5rpm to 120 rpm The viscosities of virgin polymers decreased as the shear rate increased, indicating psuedoplastic behaviour at 160°C (Figure 5.8). Shear thinning is found to be more for EVA12 compared to mPO and EVA18. Similar result was observed by Kontopoulou et al in their study on the EVA/mPO blends (14). This shows that the uncoiling of the molecular chains is most prominent in the case of EVA12. This is understandable since EVA18 is likely to have a more stable structure due to its higher polarity and hence may resist uncoiling more than that of EVA12. Shear thinning of mPO is also found to be very significant even higher than EVA12.

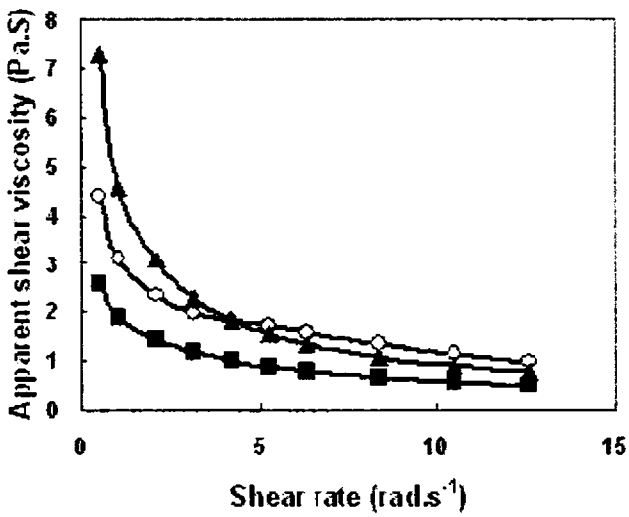


Figure 5.8 Shear rate vs Shear viscosity of the virgin polymers ○-mPO, ▲-EVA12 and ■- EVA18

5.3.2.3 Effect of Vinyl Acetate content on shear thinning

A comparison of the shear thinning effect of the blends of the both systems mPO/EVA12 and mPO/EVA18 is carried out by taking the blends having 25%EVA content form the each system. Figure 5.9 shows the variation of shear viscosity of both blend compositions with varying shear rates. The figure indicates the effect of vinyl acetate content in the blend on the viscosity variation with different shear rates. The blend mPO/EVA12 shows a higher shear thinning property compared to the blend containing EVA having 18%vinyl acetate. This indicates processing of EVA12 is comparatively easier to that of EVA18. Moreover, it has been indicated that increase in the extent of shear thinning increases melt strength and reduces susceptibility to melt fracture and draws resonance and therefore improves their processability (14). The temperature variation due to shear heating of these blends showed more or less same values at different shear rates as shown in Figure 5.10. The energy required for processing (area under the mixing curve) is also higher for for AE1225 blend compared to AE1825 is especially at higher shear rates as seen in the Figure 5.11.

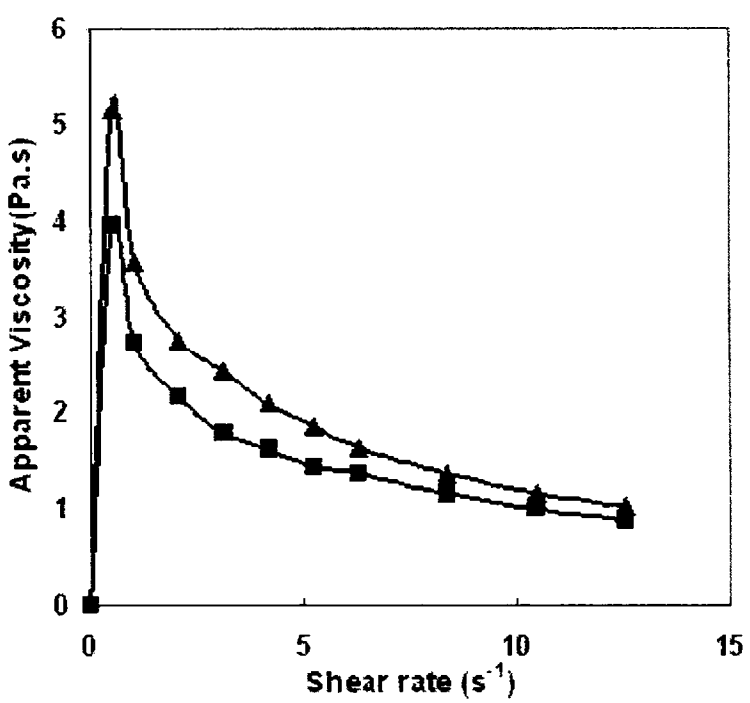


Figure 5.9 Shear rate vs. Apparent viscosity: Effect of vinyl acetate content ▲-AE1225 and ■-AE1825

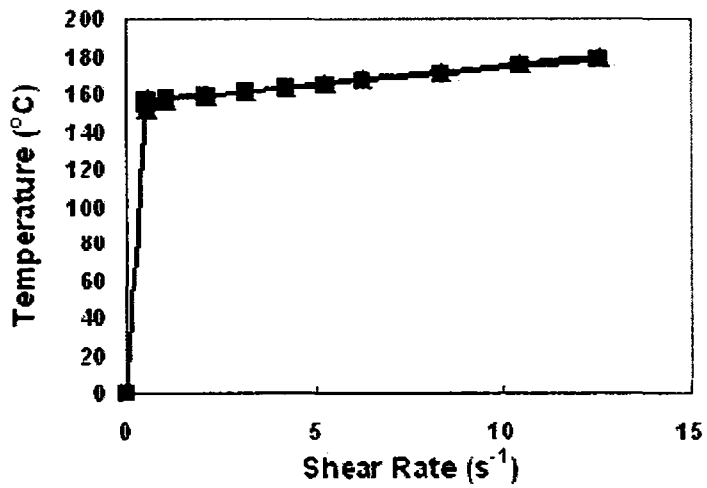


Figure 5.10 Shear rate vs. Temp: Effect of vinyl acetate content ▲-AE1225 and ■-AE1825

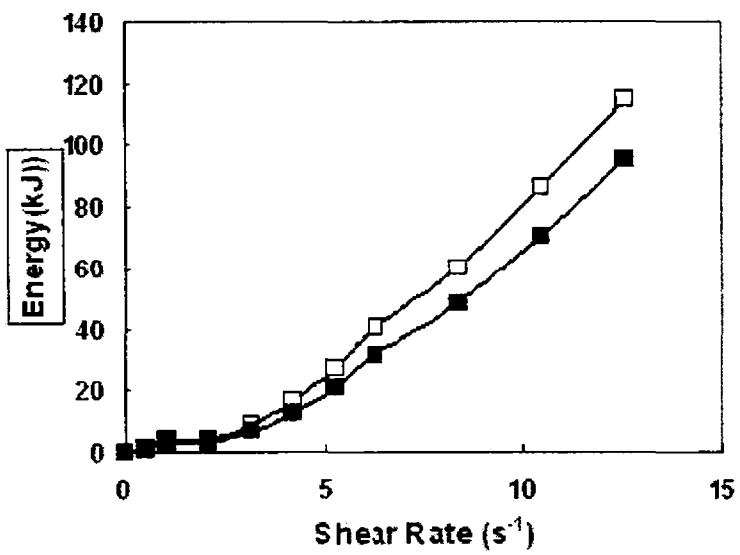


Figure 5.11 Shear rate vs energy: Effect of vinyl acetate content ▲-AE1225 and ■-AE1825

5.3.3 Thermal Studies of the Modified samples

5.3.3.1 Thermo gravimetric Analysis

The thermal degradation behaviour of mPO and the modified samples AE1225 and AE1825 is given in Figure 5.12. It can be seen that the degradation of mPO and the modified samples starts only around at 450° C. It may be inferred from figure 5.12 that the thermal degradation of EVA takes place at two distinctive temperature ranges indicates the selective volatilizarion of the functional groups. The first decomposition step involves loss of acetic acid and takes place around 240-260°C. The second decomposition step involves the cleavage of C-H bonds and takes place at a much higher temperature (>400°C). It clearly indicates that the material is stable at the processing temperature of 160°C.

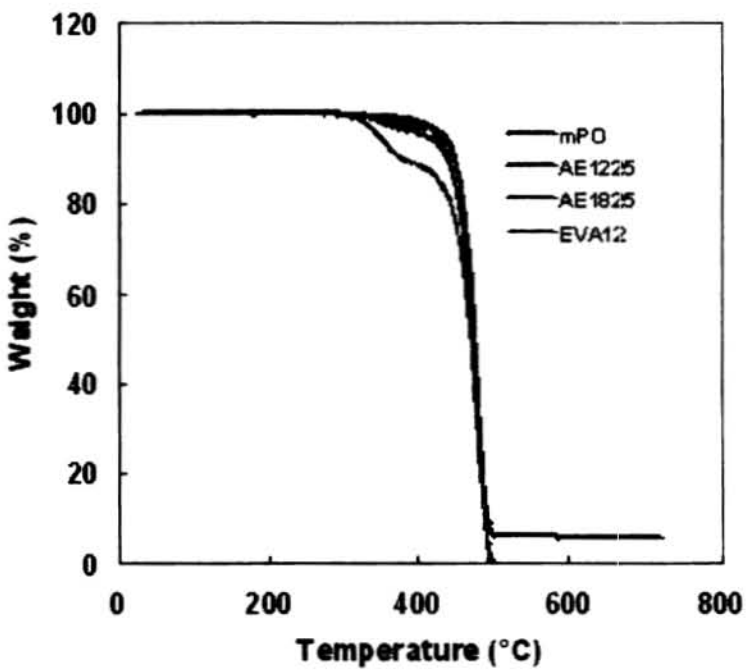


Figure 5.12 Thermal degradation profile of mPO and modified samples

5.3.3.2 Differential Scanning Calorimetric Analysis

To find the safe processing behaviour, DSC analysis of mPO, EVA, EV18 and their blends were evaluated. The DSC curves given in the Figure 5.13 show that mPO melts at 107°C, EVA12 at 96°C and EVA18 at 86°C and that the blend can be safely processed at 160°C.

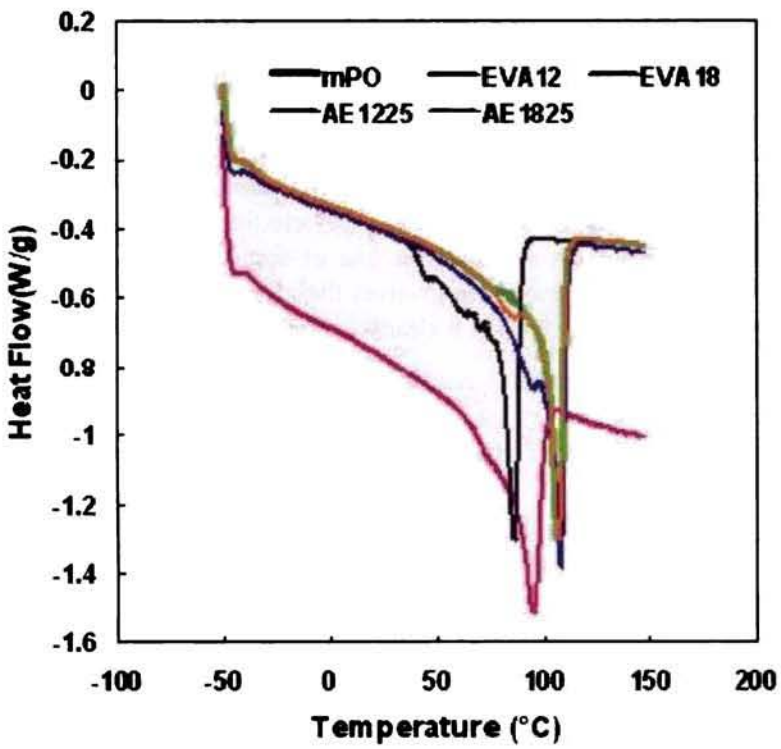


Figure 5.13 DSC curves for mPO, EVA12, EVA18 , AE1225 and AE1825

5.3.3.3 Melt Flow Index Measurements

Melt flow index values of the virgin and blended samples of mPO and EVA12 are given Table 5.1.

Table 5.1 Melt flow index of the virgin and blended samples of mPO and EVA12

Samples	*MFI (g/10min)*
mPO	3.65 ± 0.025
AE1205	3.61± 0.03
AE1215	3.42± 0.019
AE1225	3.24 ± 0.032
AE1250	3.022 ± 0.015
EVA12	2.09 ± 0.027

The melt flow index values progressively got reduced with increase in EVA12 content as seen in the Figure 5.14, which means that processing becomes marginally difficult on modification with EVA12

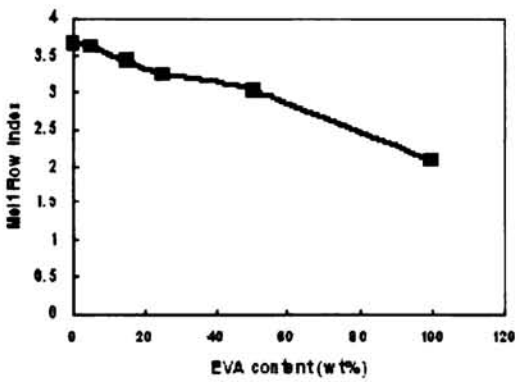


Figure 5.14 Effect of EVA content on Melt flow index of the virgin and blended samples of m-PE and EVA12

5.3.4 Mechanical Property evaluation of Blends

The stress--strain curves for the pPVC , mPO, EVA18, AE1825 and AE1850 are shown in the Figure 5.15. From the figure it can be seen mPO and mPO/EVA blends possess better mechanical characteristics than pPVC even though EVA12 and EVA18 show marginally lower tensile strengths

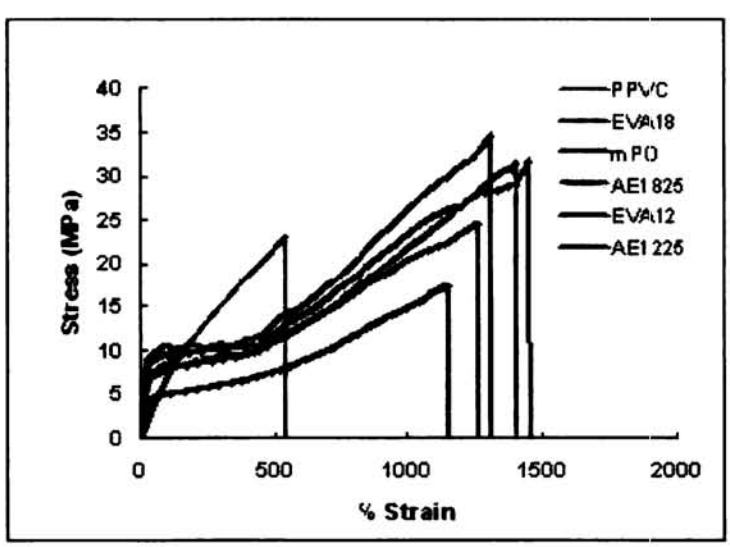


Figure 5.15 Stress –strain curves for virgin and modified samples

5.3.4.1 Effect of EVA and Vinyl Acetate contents on Tensile Strength of the Blends

The effect of EVA content on tensile strength of the blends mPO/EVA12 and mPO/EVA18 is given in Figure 5.16. There is a gradual decrease in the tensile strength of the blends with increase in EVA content as observed in both cases. The effect of vinyl acetate content on tensile strength can also be seen in the figure. A marked difference in the tensile strength is seen only in the case of virgin polymers EVA12 and EVA18 and in the case of blends there is only a marginal variation. Most of the blends are found to have a higher strength than pPVC.

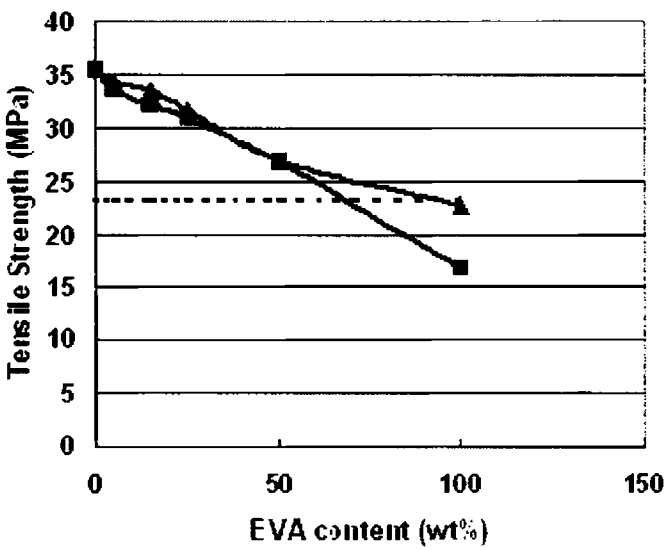


Figure 5.16 Effect of EVA and VA content on the tensile strength of the blend ▲- mPO/ EVA12■- mPO/ EVA18 blend and ----- pPVC

5.3.4.2 Effect of EVA and Vinyl Acetate contents on Percentage Elongation of the Blends

Figure 5.17 shows the effect of EVA content on percentage elongation of the blends of mPO/-EVA12 and mPO/EVA18. There is no significant variation in the percentage elongation of the blends. Similarly, the amount of vinyl acetate in the blend has no marked effect on the percentage elongation of the samples. A comparative assessment shows that all the blends of mPO/-EVA12 as well as mPO/EVA18 possess higher percentage elongation than that of pPVC.

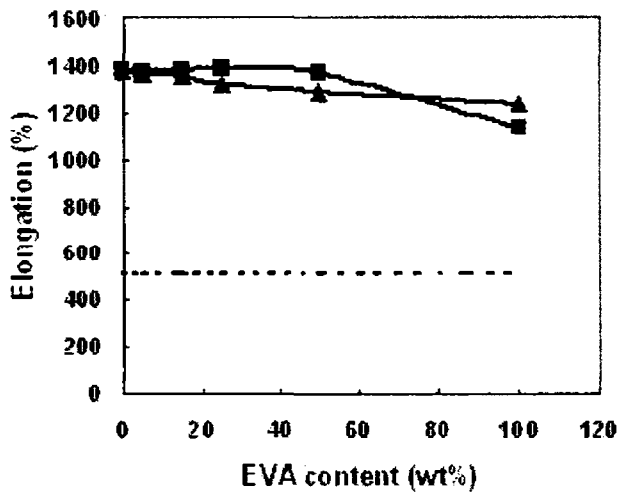


Figure 5.17 Effect of EVA and VA content on the Tensile Strength of the blend ▲- mPO/ EVA12■- mPO/ EVA18 blend and ----- pPVC

5.3.4.3 Tear strength evaluation

The general principle of the test consists of measuring the force required to completely rapture or tear the specified test piece as a continuation of the cut or nick in the test piece completely across the width of the test piece. The effects of EVA and vinyl acetate contents of the blend on tear strength are given in Figure 5.18. From the figure it is clear that the tear strength decreases with increase of EVA content. A similar trend can be seen in the case of vinyl acetate content in the blend. However, further shows that the tear strengths of all the blends are very higher than that of pPVC.

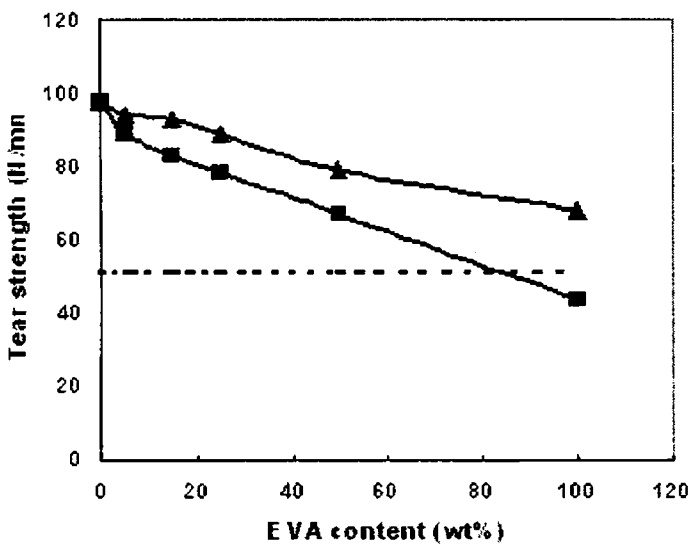


Figure 5.18 Effect of vinyl acetate content on the tear strength of mPO/ EVA blends▲- EVA12 and ■-EVA18 and ----pPVC

5.3.6 Density measurements

The results of density measurements of the blends mPO/EVA12 and mPO/EVA18 are given in tables 5.2 and 5.3 respectively. The densities obtained by linear mixing rule are also given in the table. There is not much variation between the experimental and theoretical values. The densities of the blends are lower than that of pPVC and this results in weight savings since their blends are stronger than pPVC. Assuming a constant volume the use of the various blends can result in material savings as shown in table 5.4. This gain not only reduces the product cost but also reduces the consumption of raw materials as well as the cost for the post use disposal.

Table 5.2 Density of mPO and its blends with EVA12

Samples	*Density (observed) (g/cc)*	*Density (theoretical) (g/cc)*
m-PE	0.908 ± 0.003	-
EVA12	0.931 ± 0.006	-
AE1225	0.914 ± 0.005	0.9136
AE1250	0.921 ± 0.009	0.9192

Table 5.3 Density of mPO and its blends with EVA18

Samples	*Density (observed) (g/cc)*	*Density (theoretical) (g/cc)*
m-PE	0.908 ± 0.003	-
EVA18	0.932 ± 0.006	-
AE1825	0.910 ± 0.005	0.914
AE1850	0.912 ± 0.009	0.920

Table 5.4 A comparative evaluation of percentage wt gain of the material for 100 cc of the product

Sample	*Density (gm/cc)*	*Mass for 100 cc material (m=Vxd) in gms*	*% wt gain*
pPVC	1.26	126	-
AE1225	0.914	91.4	37.86
AE1250	0.921	92.1	36.80
AE1825	0.910	91.0	38.46
AE1850	0.912	91.2	38.16

5.3.7 Water Vapour Transmission Rate

5.3.7.1 Effect of EVA content on Sp.WVTR

Figure 5.19 shows the effect of EVA12 content in the blend mPO/EVA12 on Sp. WVTR. at 23°C. Not much variation in the Sp.WVTR is observed in the blends having EVA content up to 25 wt%. A marginal increase is seen in WVTR beyond that level. This may be due to the enhanced polarity of the blends with increase in EVA content.

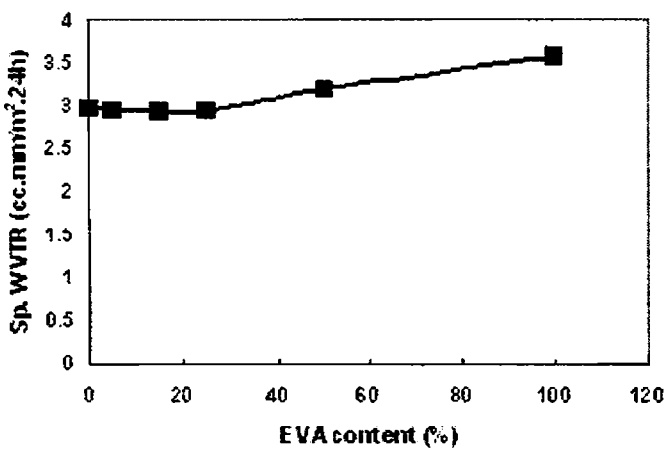


Figure 5.19 Effect of EVA content on Sp.WVTR of the blend m-PE/ EVA12 at 23°C

The effect of EVA18 content in the blend mPO/EVA18 on WVTR at 23°C is shown in the Figure 5.20. An increase in WVTR is seen with the increase of EVA content as in the case of EVA12.

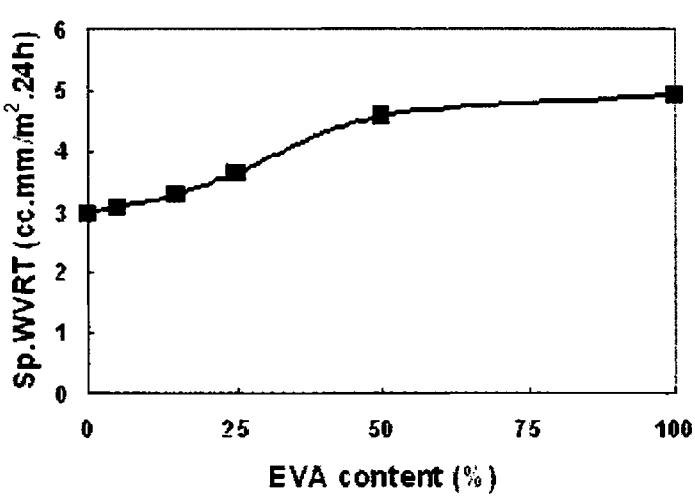


Figure: 5.20 Effect of EVA content on WVTR of m-PE/ EVA18 blend at 23°C

5.3.7.2 Effect of Temperature on Sp.WVTR

The temperature dependence of Sp.WVTR of the mPO/EVA12 blends is as shown in Figure 5.21. An increased Sp.WVTR is observed at higher temperature ranges. On comparison with pPVC and EVA12 all the blends show low Sp.WVTR. This feature may be good for the materials to be used for fluid storage application purposes.

Figure 5.22 shows the effect of temperature on the Sp.WVTR of the blends containing m-PE and EVA18. As expected, the Sp.WVTR increases with the increase of temperature.

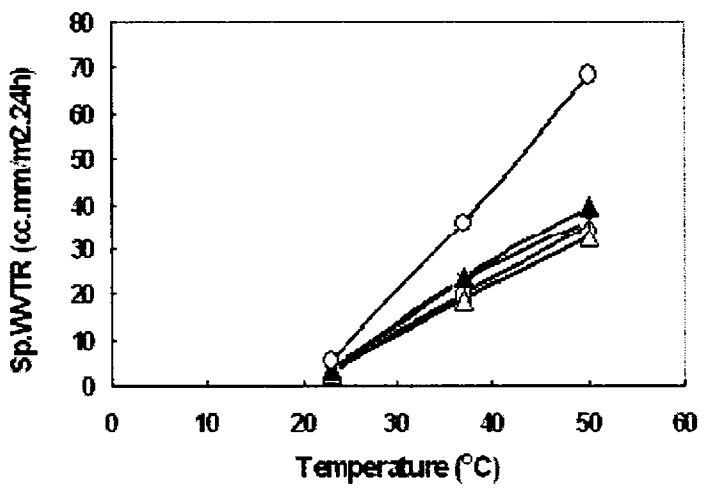


Figure. 5.21 Temperature dependence of WVTR of mPO/ EVA12 blends□-mPO, △-AE1225, -AE1250, ▲- EVA12 and ○ -pPVC

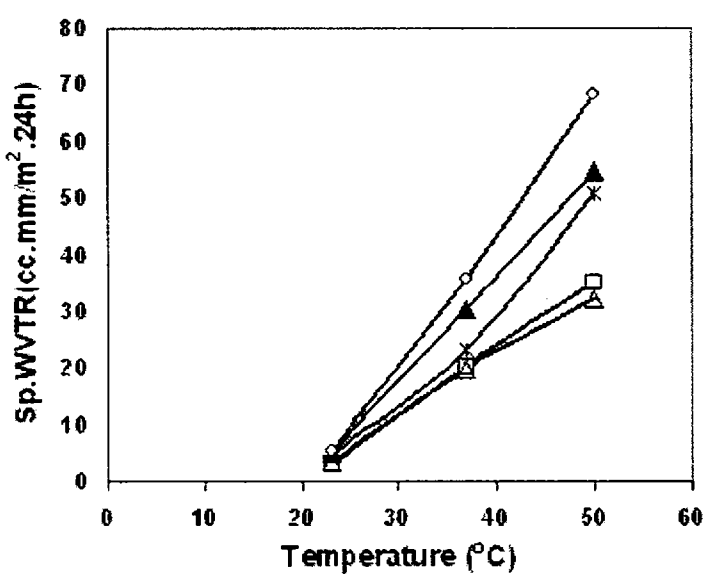


Figure. 5.22 Temperature dependence of Sp.WVTR of mPO/ EVA18 blends□-mPO, △-AE1225, -AE1250, ▲- EVA12 and ○ -pPVC

5.3.7.3 Effect of Vinyl Acetate Content on Sp.WVTR

The effect of vinyl acetate content in the blend on Sp.WVTR is shown in from the Figure5.23. An increased Sp.WVTR is observed with an increase in vinyl acetate content. Moreover, the difference is more at higher levels of EVA content. But when compared with pPVC, the Sp.-WVTRs for all the blends are less.

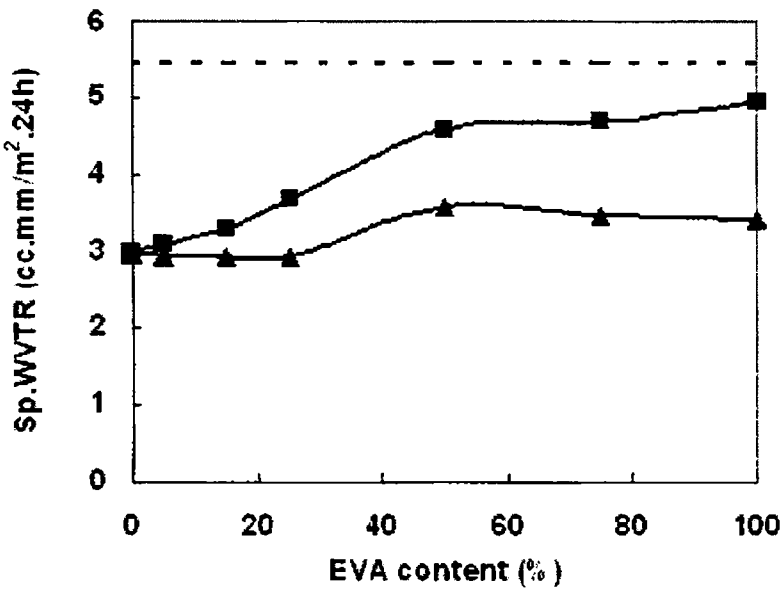


Figure 5.23 Effect of vinyl acetate content in the blend on Sp. WVTR (▲) EVA12 and (■) EVA18 ----pPVC

5.3.8 Swelling Studies

Figure 5.24 shows the effect of vinyl acetate content on the swelling of the samples in water. Very negligible amount of water intake is seen in both the blends. However the blend containing EVA18 shows slightly higher values than that of EVA12. This due to the increased level of polar acetate groups in the case of EVA18.

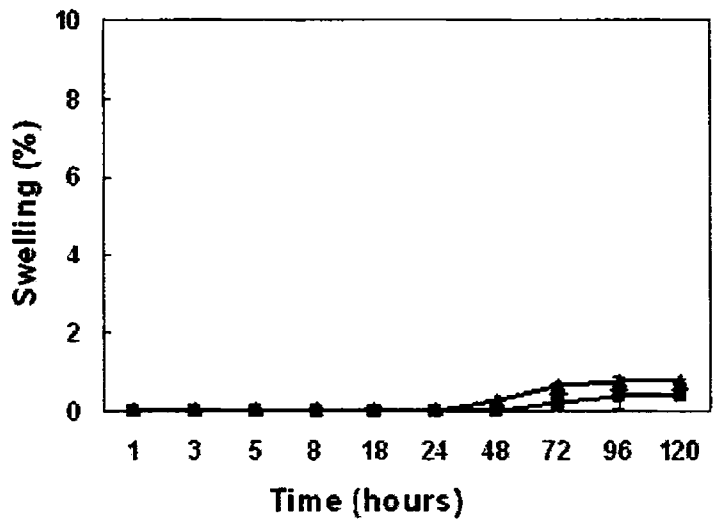


Figure. 5.24 Effect of vinyl acetate content on percentage swelling -AE1272 and ▲-AE1872

5.3.9 Water Contact angle measurements

Figures 5.25 & 5.26 show the contact angle of the blends mPO/EVA12 and mPO/EVA18 respectively. One interesting fact observed is that the addition of EVA drastically decreased the water contact angles in all the cases. The decrease of the contact angles may be due to the polar acetate groups present on the surface of the samples. The increase of EVA content in the blend does not seem to affect the contact angle significantly.

Surface energy characteristics of the material are important criterion in the biomaterial assessment. Low water contact angle favours less adhesion of cells and proteins onto the surface of the material. The material, which has water contact angle less than 50^{o} tends to absorb high amount of water and is not advisable for the materials intended to use for fluid storage applications. Too high a water contact angle indicate that the material is hydrophobic and not good for the above-mentioned purposes as it can damage the cells due to the adhesive force. In the case of PVC bags, the water contact angle is usually found to be in range of $60\text{-}70^{o}$.

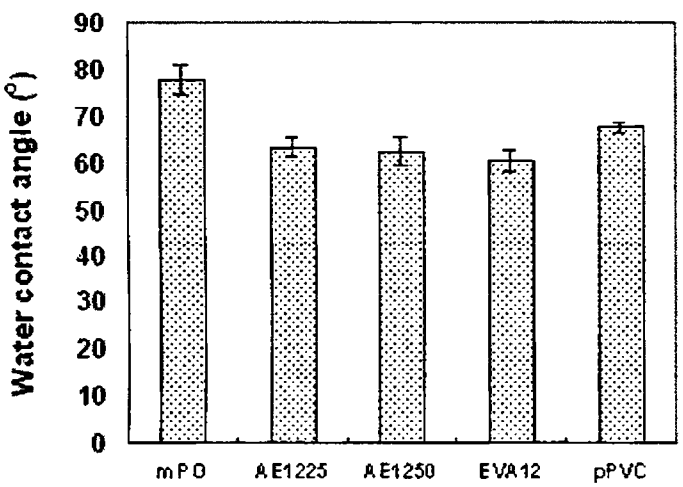


Figure5.25 Water contact angle of the virgin and blend samples mPO/ EVA12

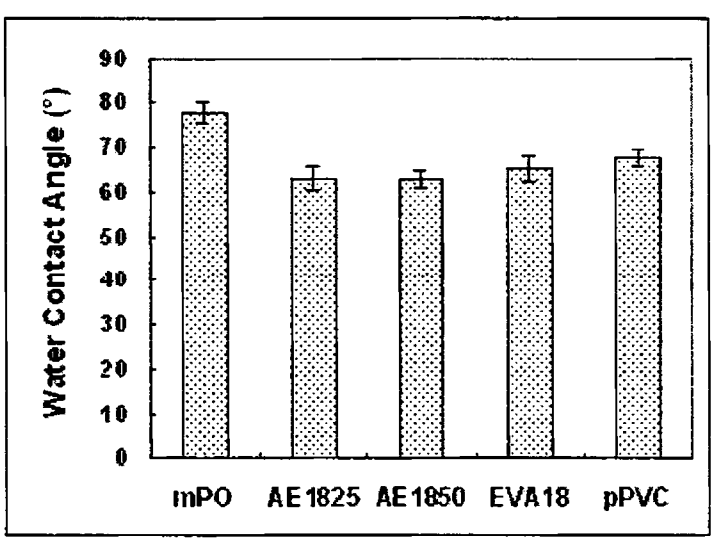


Figure5.26 Water contact angle of the virgin and blend samples mPO/ EVA18

5.3.10 Hardness studies

Shore A harness test was carried out on the virgin as well as the blend compositions. The comparison of the hardness of the blends of mPO with EVA12 is given in the Figure 5.27. The results show that hardness of the blends is not much difference from mPO alone.

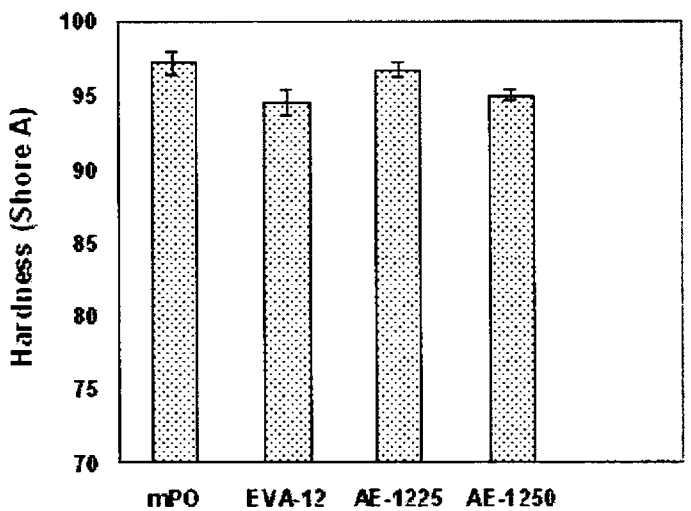


Figure 5.27 Comparison of Hardness (Shore A) of the blend of m-PE/ EVA12 with the virgin polymers

5.3.11 Transparency Test

The results of the transparency test conducted on samples are given in the table 5.5. It shows that transparency of the blends not affected much due to the modification of mPO with EVA.

Table 5.5 Percentage transmittance of the samples at 540nm

Sample	*Transmittance (%)*
m-PE	70
EVA12	72
AE1225	69
AE1250	69
EVA18	76
AE1825	68
AE1850	70
pPVC	65

5.3.12 Gas Permeability Studies

Gas-permeability is one of the important characteristics of blood component storage containers. It is essential so that the living cells of the blood component, such as red blood cells and platelets, can exchange oxygen and carbon dioxide. This allows for the extended viability of the living blood component and longer storage times. Gas permeability was carried out at three different temperatures (10, 25, 40°C) with oxygen and carbon dioxide gases.

Figures 5.28 and 5.29 show the oxygen and carbon dioxide permeabilities of the blend containing EVA12 respectively. The figures indicate that the blending with EVA increased the permeability of both gases. It further shows that the permeability increases with increase of EVA content. The increase in temperature enhances the permeability of both gases as expected.

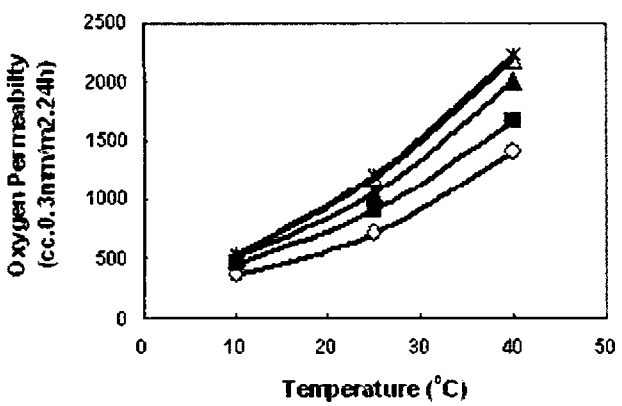


Figure 5.28 Oxygen permeability of mPO and its blends with EVA12. o-mPO, ■-AE1215, ▲- AE1225, △-AE1250 and -EVA12

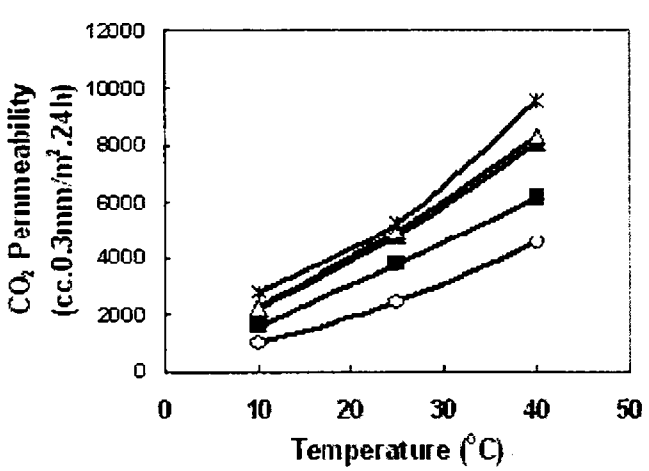


Figure 5.29 Carbon dioxide permeability of mPO and its blends with EVA12. o-mPO, ■-AE1215, ▲- AE1225, △-AE1250 and -EVA12

Similar results of gas permeability are seen in the case of the blend having EVA18. The results of oxygen and carbon dioxide permeabilities for the blend mPO/EVA18 are given in the figure5.30 and figure 5.31 respectively.

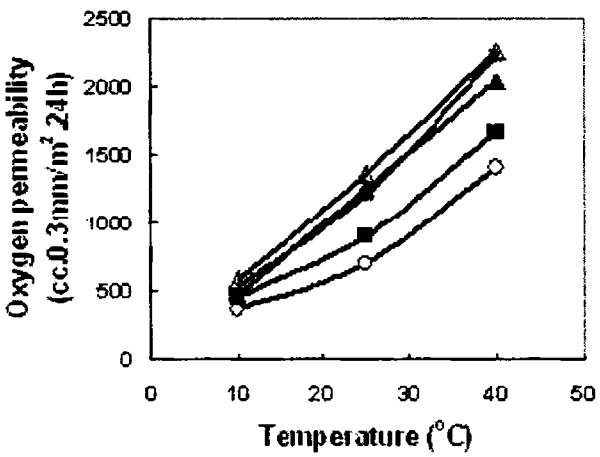


Figure 5.30 Oxygen permeability of m-PO and its blends with EVA18. O-mPO, ■-AE1815, ▲- AE1825, △-AE1850 and -EVA18

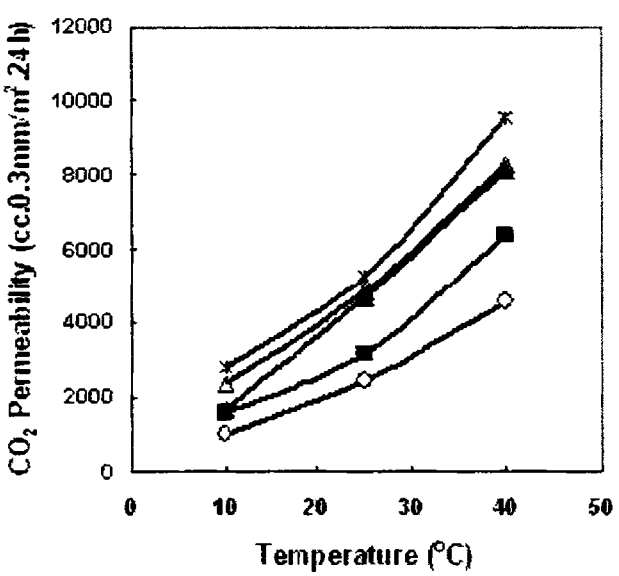


Figure 5.31 Carbon dioxide permeability of m-PO and its blends with EVA18. O-mPO, ■-AE1815, ▲- AE1825, △-AE1850 and -EVA18

The container for body fluid storage application should have good oxygen as well as carbon dioxide permeabilities. So the ratio of the permeability of CO_2 to O_2 is very critical. The conventional platelet bags have the CO_2 to O_2 permeability ratio around or more than 4. CO_2 to O_2 permeability ratio was calculated for all the blends and are given in Table5.6

Table 5.6 Carbon dioxide to oxygen ratio for the blends containing EVA12 and EVA18

Samples	*Temperature* (^0C)	*Permeability* CO_2	O_2	CO_2/O_2 *ratio*
	10	1003	365	2.75
mPE	25	2427	703	3.45
	40	4551	1410	3.23
	10	1575	457	3.45
AE1215	25	3780	902	4.19
	40	6170	1668	3.70
	10	2198	510	4.31
AE1225	25	4800	1053	4.56
	40	8082	2001	4.04
	10	2242	524	4.27
AE1250	25	4757	1185	4.01
	40	8299	2186	3.79
	10	1575	457	3.45
AE1815	25	3160	902	3.50
	40	6375	1668	3.82
	10	1675	474	3.53
AE1825	25	4681	1253	3.74
	40	8115	2043	3.97
	10	2361	584	4.04
AE1850	25	4842	1361	3.55
	40	8279	2264	3.66
	10	1937	534	3.63
PPVC	25	5070	1043	4.86
	40	8341	2250	3.70

From the table the CO_2 to O_2 ratio is found to be acceptable and consistent for the blend AE1225 and there is not much variation with temperature.

A comparative study of the oxygen and carbon dioxide permeability of the most suitable blend AE1225 was carried out with that of pPVC at three different temperatures. The results for oxygen and carbon dioxide permeability are given in the Figures 5.32 & 5.33. The oxygen and carbon dioxide permeabilities of the blend AE1225 are found to be more or less equal to those of pPVC especially in the storage temperature of the blood and blood components. Moreover, the ratio of CO_2 to O_2 ratio is also found to be better than pPVC

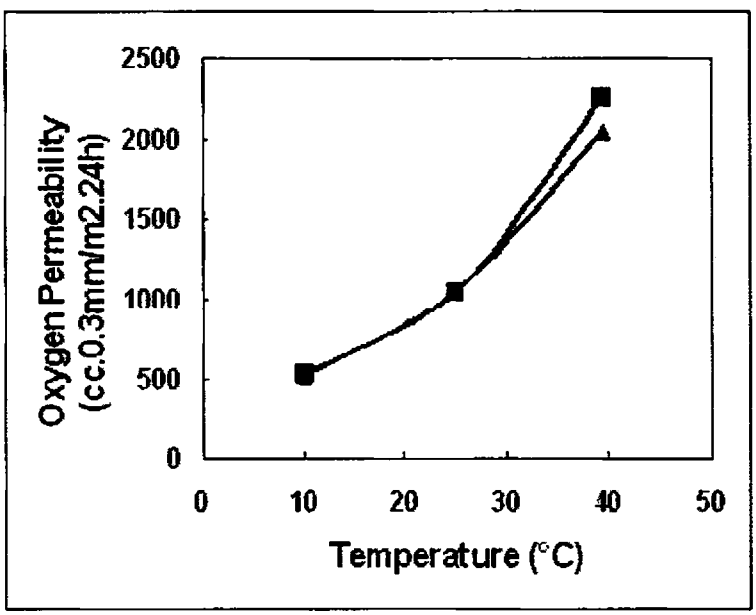


Figure 5.32 Comparison of oxygen permeability of AE1225 with pPVC at 25°C. ■-pPVC and ▲-AE1225

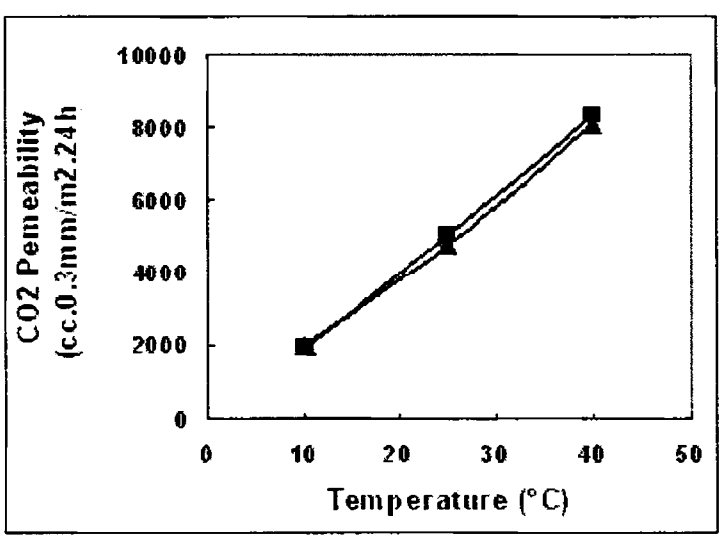


Figure 5.33 Comparison of carbon dioxide permeability of AE1225 with pPVC at 25°C. ■-pPVC and ▲-AE1225

5.3.13 Phase Morphology

The micrographs shown in the figures 5.34 (a), (b) and (c) are the SEM photographs of the cryogenically fractured surfaces of mPO, EVA12 and the blend AE1225 respectively. The fracture surfaces of mPO, EVA12 and their blend are found to be more or less the same indicating a stable behaviour of the blend.

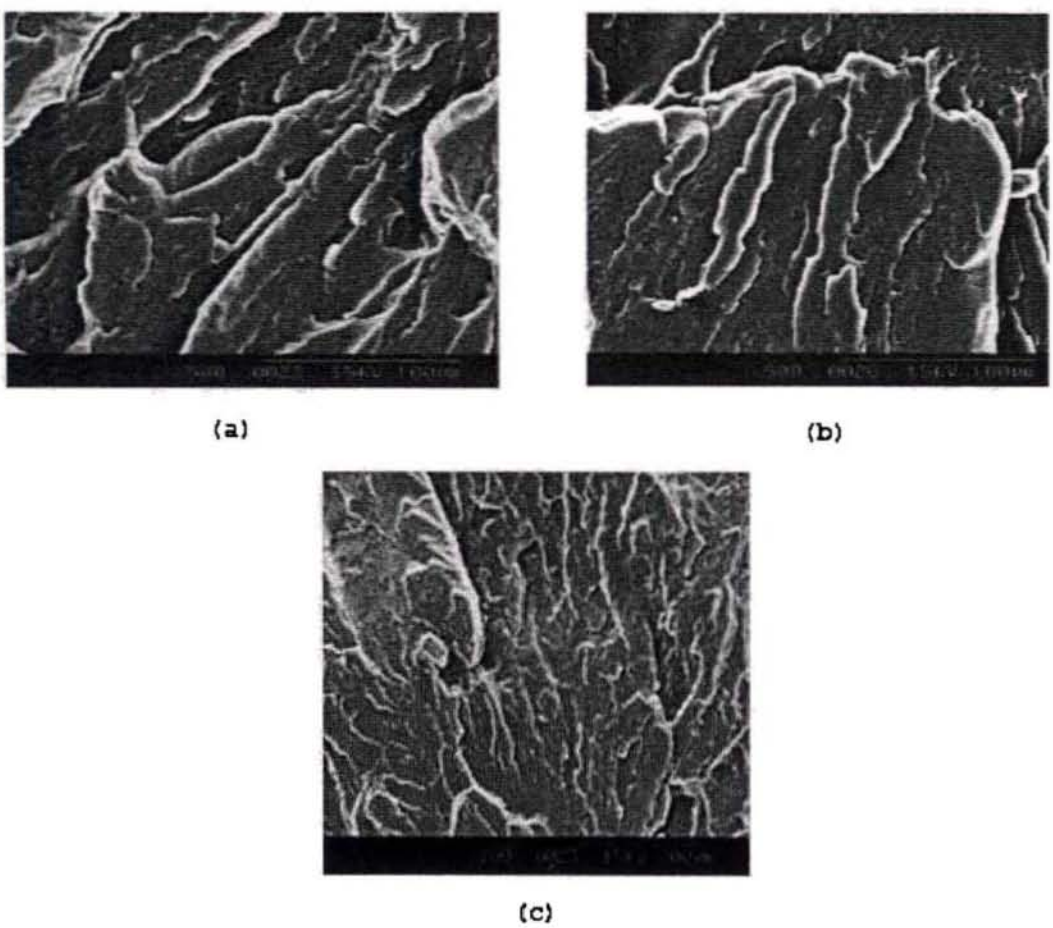


Figure 5.34. Scanning electron micrograph of cryogenically fractured surfaces of (a) mPO, (b) EVA12 and (c) AE1225.

To see the phase morphology the etched surfaces of cryogenically fractured samples were observed in scanning electron microscope and the micrographs for mPO, AE1225 and AE1250 are shown in figures 5.35 (a), (b) and (c) respectively. From the figure it is clearly seen that EVA phase is completely etched by dichloroethane and that the EVA phase is uniformly distributed in the blend. For the blends containing 25% and 50% EVA mPO is found to form the continuous phase and the EVA phase is distributed more uniformly as fine particles in the case of the former accounting for its good mechanical strength.

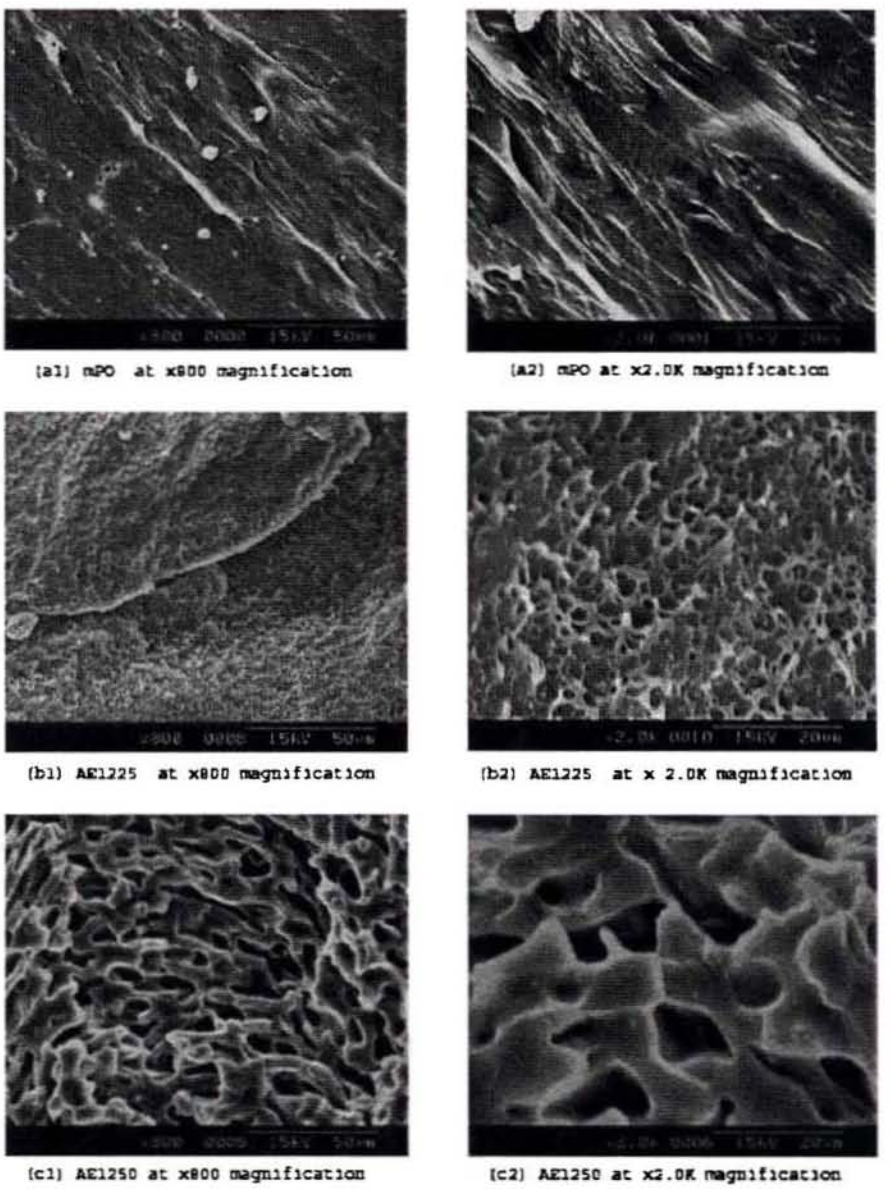


Figure 5.35: EVA etched out samples (a)mPO, (b)AE1225 and (c)AE1250

5. 3.14 Effect of Sterilization

Bio-medical products undergo sterilization procedure before its clinical use. Sterilization of the blood component storage container is also of great importance (15). The sterilization by radiation may cause material degradation or property variation. So the effect of sterilization is a point worth studying. The properties of the material after sterilization are determined and compared with the properties of the materials before sterilization. The degradation of EVA can be assessed by checking the pH of the extracts in distilled water before and after the sterilization. It was observed that irradiation with gamma ray of .5 Mrad dose range did not effect the pH of the extract from EVA copolymer.

5.3.14.1 Tensile strength

Figures 5.35 &5.36 show the effect of sterilization on the tensile properties of the blends containing EVA12 and EVA18 respectively. It can be seen that there is no much deterioration the tensile strength of the samples after sterilization.

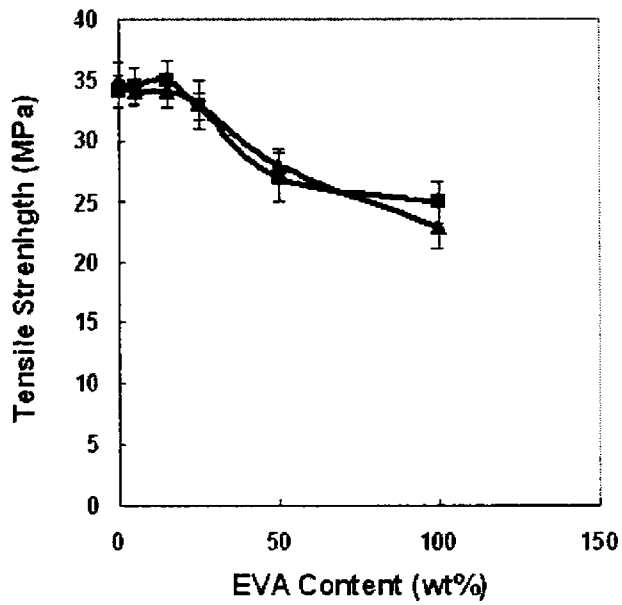


Figure 5.35 Tensile strength of the blends containing EVA12, ▲-before and ■ - after sterilization

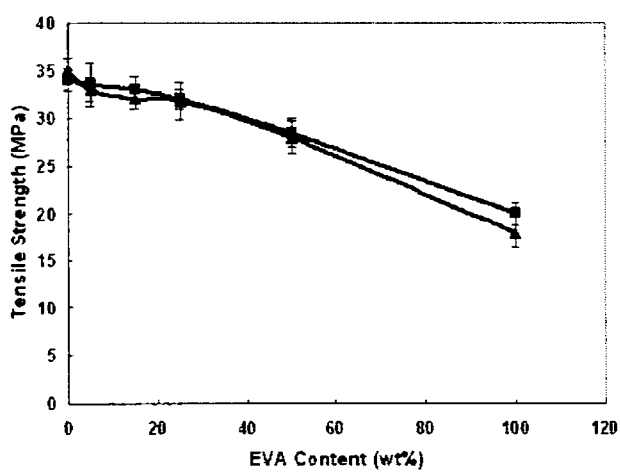


Figure 5.36 Tensile strength of the blends containing EVA18, ▲-before and ■ - after sterilization

5.3.14.2 Percentage Elongation

Figures 5.37 & 5.38 show that the percentage elongation of the samples are not altered significantly by sterilization.

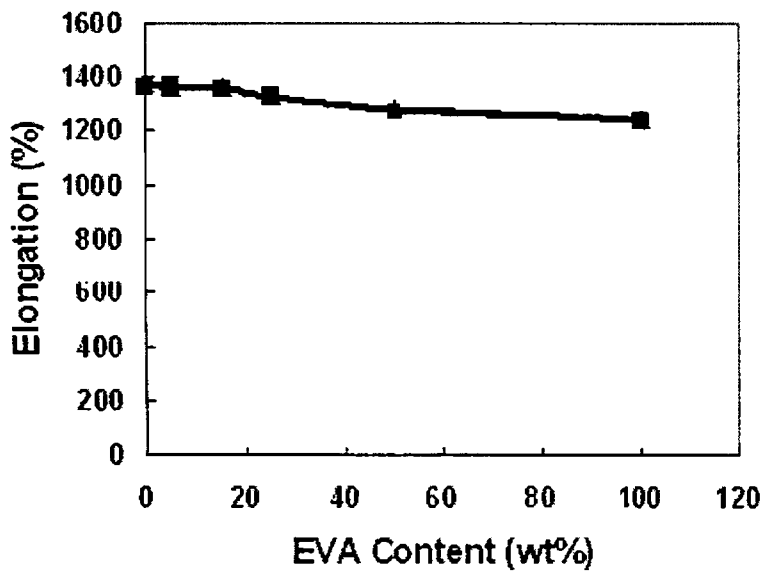


Figure 5.37 Percentage elongation of the blends containing EVA12,▲-before and ■ - after sterilization

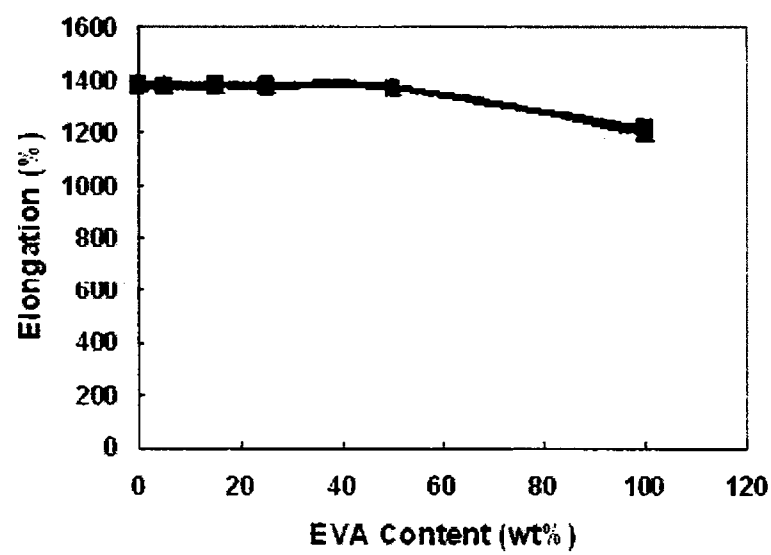


Figure 5.38 Percentage elongation of the blends containing EVA18,▲-before and ■ - after sterilization

5.3.14.3 Tear Strength

No much variation in the tear strength of the blends is observed as in Figures 5.39 & 5. 40

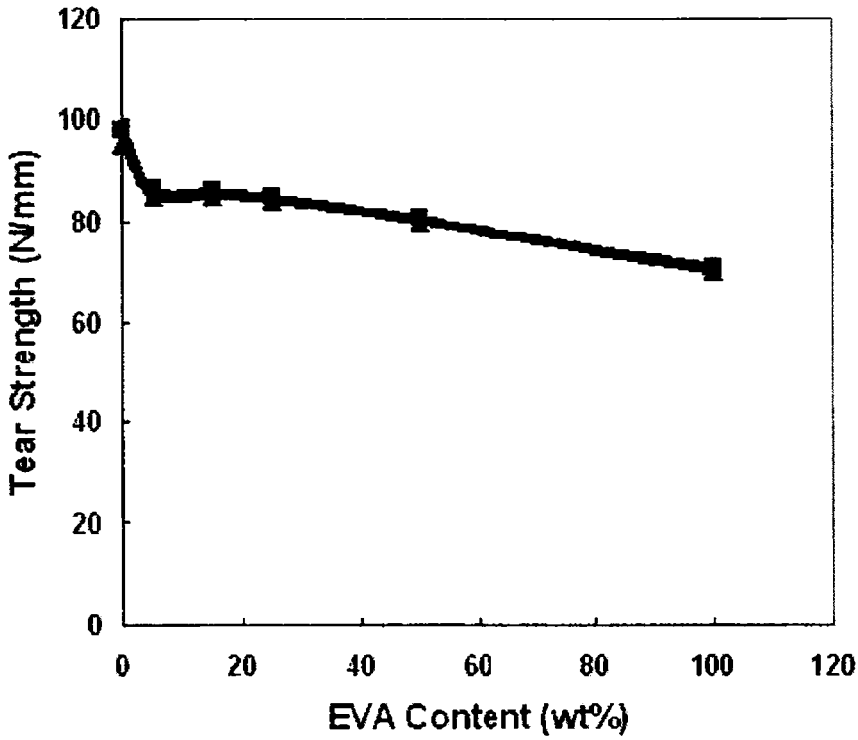


Figure 5.39 Tear strength of the blends containing EVA12,▲-before and ■ - after sterilization

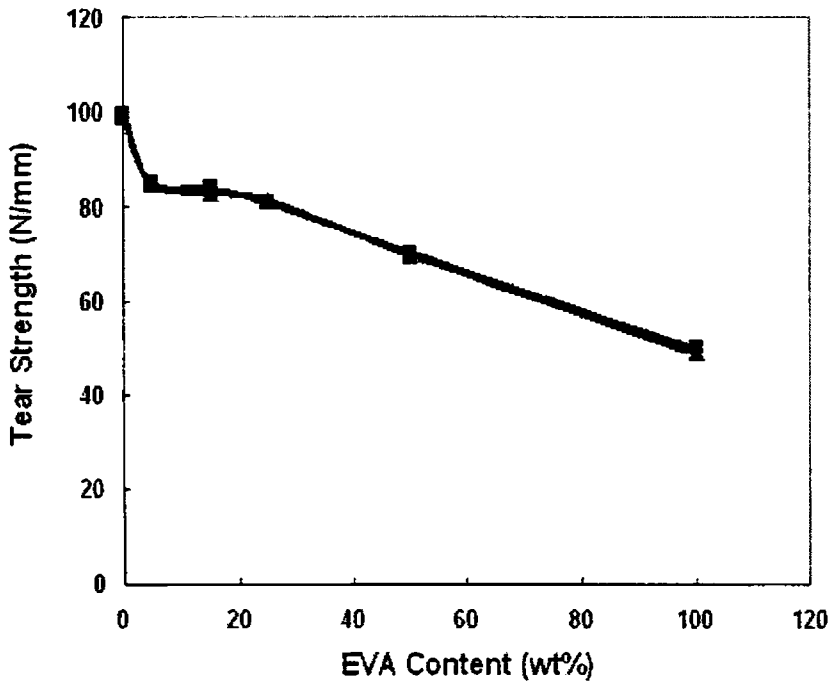


Figure 5.40 Tear strength of the blends containing EVA18,▲-before and ■ - after sterilization

5.3.14.4 Sp.Water Vapour Transmission Rate

No significant variation in the Sp.WVTR is observed for the blends due to sterilization as indicated in Figures 5.41 & 5.42

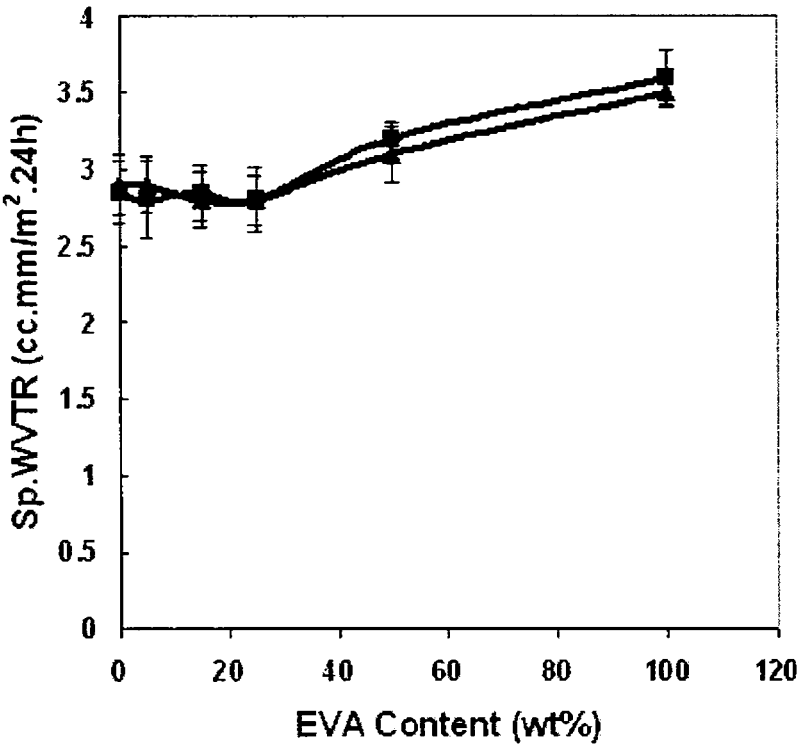


Figure 5.41 Sp.WVTR of the blends containing EVA12,▲-before and ■ - after sterilization

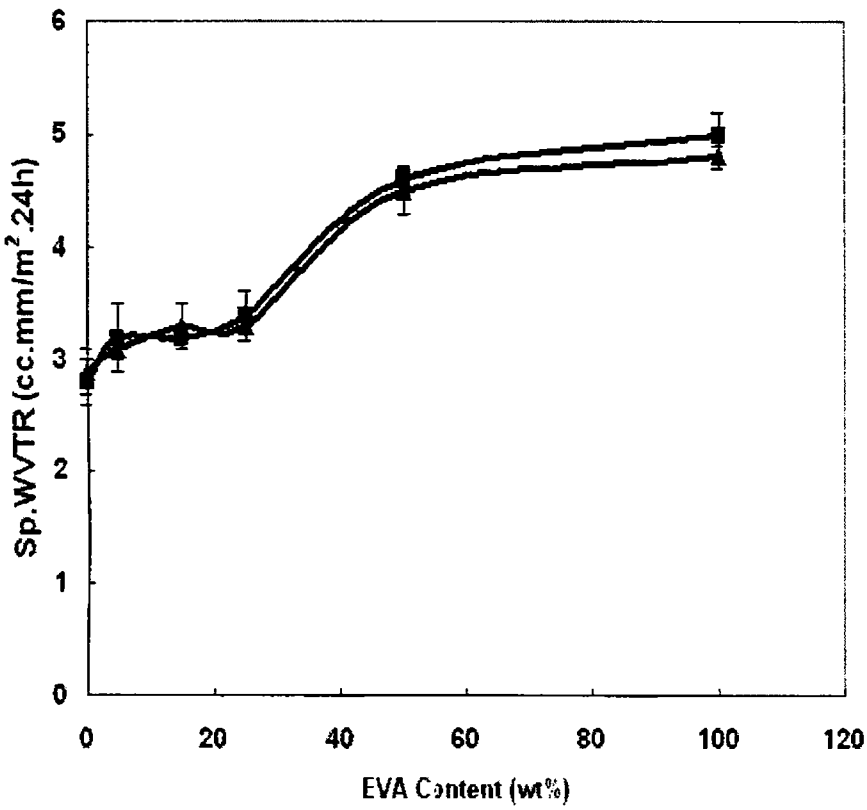


Figure 5.42 Sp.WVTR of the blends containing EVA18,▲-before and ■ - after sterilization

5.3.14.5 Transparency

There is no much variation in percentage transmittance of the blends before and after sterilization as evidenced in the Table 5.7

Table 5.7 Percentage Transmittance of the samples before and after sterilization

	Transmittance (%)	
Sample	Before radiation	After radiation
m-PE	70	70
EVA12	72	72
AE 1225	69	70
AE 1250	69	70
EVA18	76	75
AE 1825	68	69
AE 1850	70	70

5.3.14.6 Water Contact Angles

The Figures 5.43 & 5.44 show the water contact angles of the blends containing EVA12 and EVA18 respectively before and after sterilization. There is no effect of sterilization on water contact angle as can be seen from the figures. This indicates that there may not be any no change occurring during sterilizationin the surface characteristics of the samples.

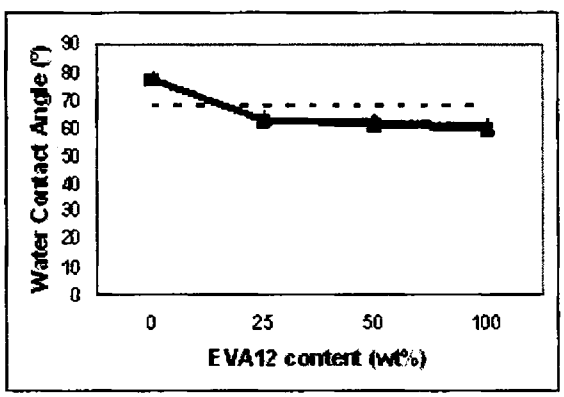
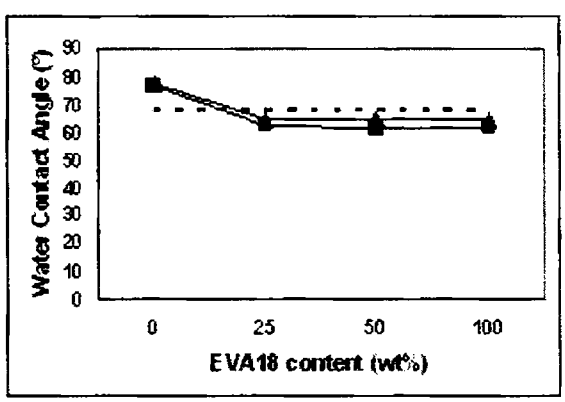


Figure 5.43 Water contact angles of the blends containing EVA12 ▲) Before and ■) after sterilization and ---pPVC



Picture 1.13. Figure 5.44 Water contact angles of the blends containing EVA18 ▲) Before and ■) after sterilization and ----pPVC

5.3.14.7 Gas permeability

Table 5.8 shows the effect of sterilization on the gas permeability of the blend AE1225. The permeability values of the sample show no significant variation before and after sterilization.

Sample	Temp-erature	Before Radiation			After Radiation		
		CO_2	O_2	CO_2/O_2	CO_2	O_2	CO_2/O_2
AE1225	10	1998	490	**4.077**	1950	485	**4.020**
	25	4480	1053	**4.255**	4445	1010	**4.401**
	40	7482	1864	**4.014**	7410	1835	**4.039**

Table 5.8 Oxygen and carbon dioxide permeability as well as CO2/ O2 the of the blend AE1225 before and after sterilization**Figure 1.32.**

5.3.14.8 pH of the extract of the material

On of the degradation product of EVA is acetic acid and the possibility of EVA degradation due γ-radiation can be assessed by checking the pH of the extract of the material before and after radiation. The pH of the extract obtained by keeping the samples before and after γ radiation in deionized water for 72 hours at 23±2°C is measured and the results obtained are shown in the table 5.9. It was observed that irradiation with γ-radiation of .5 Mrad dose range did not effect the pH of the extract from EVA copolymer. According to Portnoy and Domine the metallocene plastomers have a high level of radiation tolerance and usually do not discolour after the typical 25 kGy (0.5Mrad) of sterilization gamma radiation (16).

Table 5.9 pH of the samples before and after γ radiation

Samples	pH	
	Before irradiation	After irradiation
Control (H_2O)	6.85 ± 0.003	-
mPO	6.74± 0.004	6.81± 0.006
AE1225	6.68± 0.013	6.72± 0.008
AE1250	6.81± 0.002	6.79± 0.010
AE1825	6.69± 0.011	6.75± 0.009
AE1850	6.78± 0.012	6.86± 0.012

5.4 Conclusion

From the results obtained it can be concluded that mPO can be modified with EVA. The evaluation of the properties of the materials after modification shows that the properties of the blends are better than those of pPVC, used as a control in this study. Marginal decrease in contact angle, lower water permeability and improved gas permeability may give these blends an edge over pPVC in the manufacture of blood or blood component storage containers. The higher gas permeability, CO_2/O_2 ratio, better surface energy characteristics and transparency make the blend AE1225 a better candidate than pPVC. Even though, steam sterilization cannot practical for these blends, the material shows good stability against gamma radiation sterilization.

5.5 References

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Chapter 6 BIOLOGICAL EVALUATION OF MODIFIED METALLOCENE POLYOLEFIN

6

BIOLOGICAL EVALUATION OF MODIFIED METALLOCENE POLYOLEFIN

6.1 Introduction

The term biological performance is related to the interaction between material and the living system. It has two important divisions i) host response and ii) material response. The traditional approach has been to define biological performance in terms of biocompatibility (host response). The issue of biocompatibility rises from recognition of the profound differences between living tissues and nonliving materials. The label "biocompatible" suggests that the material described displays universally "good" or harmonious behaviour in contact with the tissue and body fluids. Biocompatibility refers essentially to the effect of the material on the biological system. Since there is a broad range of materials available and only a very small percentage has been used in the biological environments, there has been a continual need for a quick screening method that can be used in vitro- outside the body. These can be divided into two classes

i) Cytotoxicity tests methods

ii) Blood contact methods (for blood contact applications)

With each of these classes there is a wide variety of tests and variations of test methods. Some variations are traditional for particular applications, while others are the practice of a particular laboratory.

The need of finding acceptable material for use in contact with the blood is of great important to the present day clinical practice. The difficulty in finding acceptable materials reflects the complex nature of blood-material interactions, which are influenced by properties of the material. Critical biocompatibility evaluation is an essential step in the development of any material intended for biomedical applications. The performance and the biological response of biomaterials must be evaluated to determine whether their compatibility and functionality are suitable for application in physiological systems. This implies the utilization of standard practices, which

recommend generic biological test methods for materials and devices according to end-use applications. These test protocols are intended to be applied to materials and medical devices for human use and recommend sufficient biological testing to establish a reasonable level of confidence concerning the response to a given material or device to a living organism, as well as guidance in selecting the proper procedures to be carried out for the screening of new or modified materials.

Biocompatibility evaluation of medical devices involves testing either the material itself or an extract from it, or both, depending on the nature of the end-use application. For the convenience of assessment, the tests are categorized into (1) in vitro screening tests, followed by (2) in vivo toxicological/biocompatibility tests. The screening tests check the preliminary safety with respect to cells/blood and are performed regardless of the final use of the materials. In vitro cell culture cytotoxicity evaluation on established cell lines is the common screening test employed to assess the acute toxicity effects of material under study. A material passes this test only if acute toxicity effects are not recorded; further tests are then done on the material to assess the toxicological/biological response by making use of suitable animal models. In vitro test methods are usually quicker and less costly than in vivo methods and do not require the use of animals.

The International Organization for Standardization (ISO) recommends a battery of biocompatibility studies to be carried out on all medical devices in accordance with their end-use applications. For example, for a material intended for blood or blood component storage applications, the following battery of tests have to be done based on the guidelines/protocols prepared by ISO-10993 (1)

1. Cell culture cytotoxicity
2. Cell adhesion studies
3. Haemolysis assay
4. Clotting time test

The biological performance of the modified metallocene polyolefin with potential future use as blood and blood component storage containers is analysed in this chapter by following standard practices for generic biological test methods for materials and devices. Mechanical and permeability property evaluation of the mPO-EVA blends (chapter 5) showed that the blends, prepared from mPO and EVA12, were significantly superior to the other compositions studied. The most suitable composition, namely AE1225 was selected for biological evaluation.

Biological evaluation studies such as cell culture cytotoxicity, cell adhesion, haemolysis and clotting time were carried out to gauge the biological performance of these blends.

6.2 Experimental

In vitro cell culture cytotoxicity of AE1225 and AE1255, the two modified samples of mPO were evaluated by direct contact method and test on extract method as per ISO 10993-5 (1999) u sing L929 mammalian fibroblast cell lines. The blood compatibility of the modified sample (AE1225) was evaluated by platelet and leukocytes adhesion studies, clotting time tests and haemolysis assay. In the clotting time test the kinetics of thrombus formation were determined using fresh human blood according to Xianghuai et al.(2).

6.3 Results and Discussion

6.3.1 In Vitro Cell Culture Cytotoxicity

Cytotoxicity is the ability of a material or substance to produce a toxic or cellular effect with a deviation from normal morphology and functionality (2). This testing is a rapid, standardized, sensitive, and inexpensive means to determine whether a material contains significant quantities of biologically harmful extractables. The cytotoxicity tests essentially consist of the assessment of cell morphology and viability when the cells come in contact with either the material or the extract of the material. The cells lysed or injured by the material or the extract of the material are examined. Generally, established mammalian cell line is used for the study. The L929 (mouse fibroblasts) have become the most commonly used cell line because they are easily cultured and scored. They also represent a cell line capable of being perpetually subcultured and have been shown to be among the preferred cell types for use in biocompatibility test procedures in vitro (3). The international standards compiled as ISO 10993, and the FDA blue book memorandum (#G95-1) that is based on ISO10993-1, address the critical issue of ensuring device biocompatibility by identifying several types of tests for use in selecting device materials. Required for all types of devices, cellular toxicity testing is covered in ISO 10993-5: "Tests for Cytotoxicity—In Vitro Methods" (4) This standard presents a number of test methods designed to evaluate the acute adverse biological effects of extractables from medical device materials.

The response of AE1225 and AE1250 to a culture of L929 cells is shown in figure 6.1 (a & b).

It can be seen from the figures that the typical spindle shape morphology of L929 was retained even after 24 h of contact with AE1225 and AE1250. Similar results were obtained for the test performed on the extract of the blend materials (Figure 6.1c&d). The results from the in vitro cell culture cytotoxicity showed that both AE1225 and AE1250 were non-cytotoxic to L929 cell line.

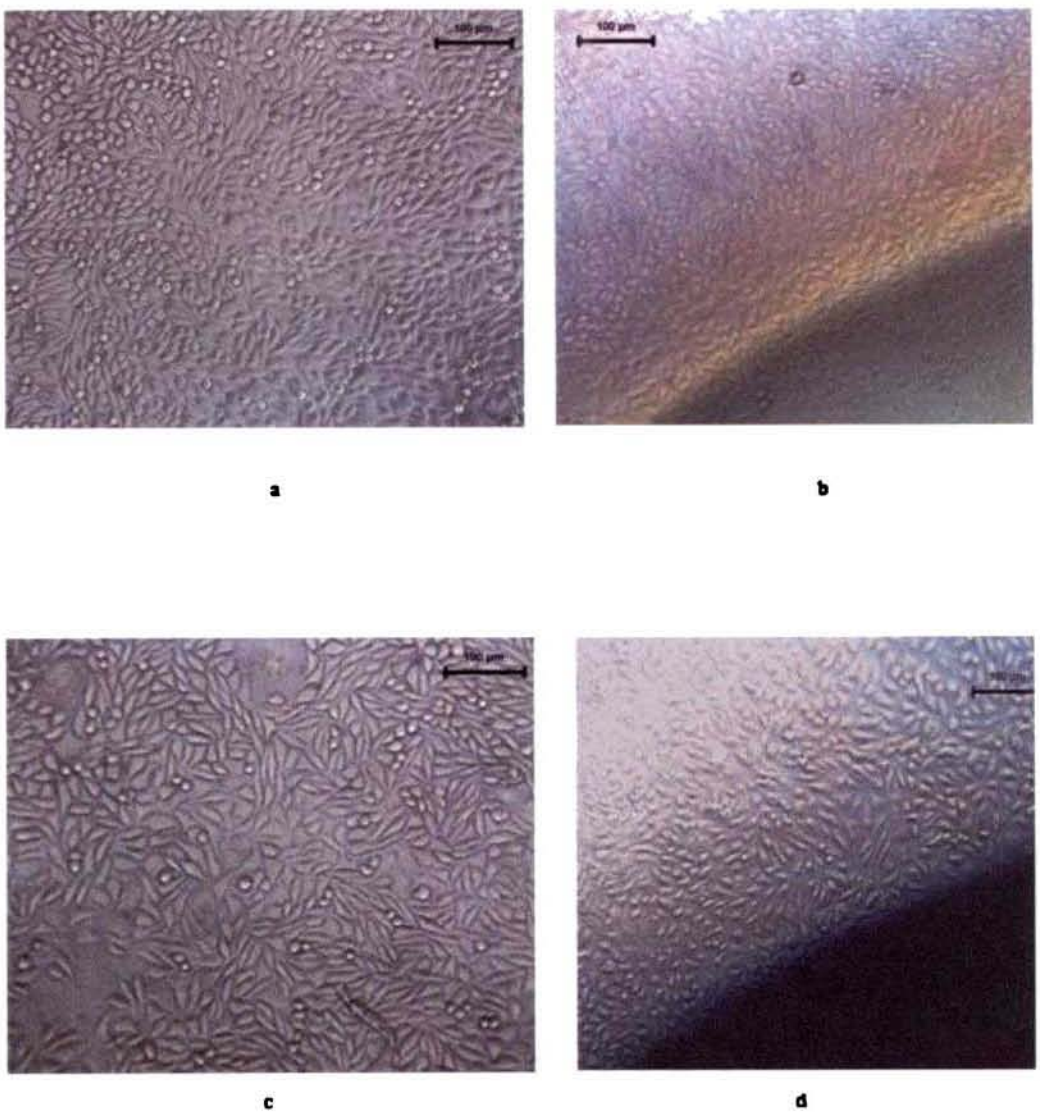
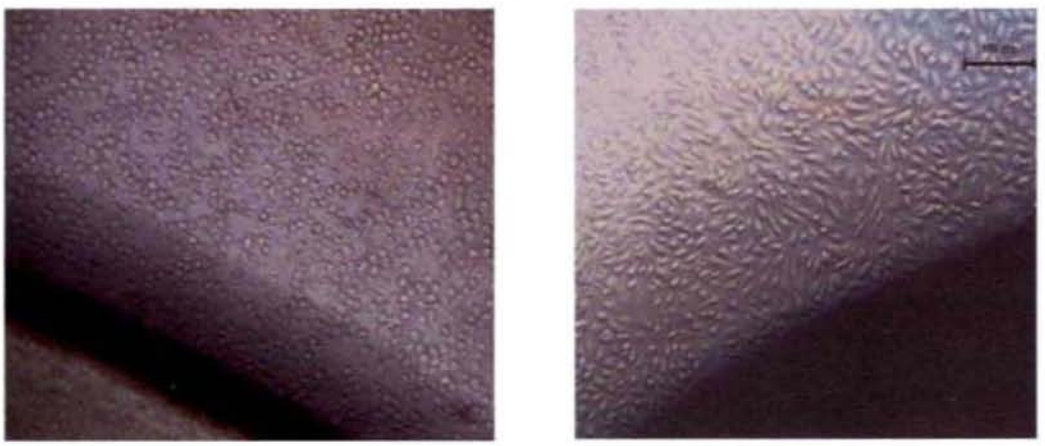


Figure 6.1 L929 cells incubated with (a) AE1272, (b) AE1255, (c) extract from AE1272 and (d) extract from AE1255 over 24 h.

Figure 6.2 (a & b) shows the shape of the cells in a positive and negative cytotoxicity control samples reaspectively. A positive cytotoxicity test result can be taken as an early warning sign

that a material contains one or more extractable substances that can be of clinical importance. In such cases, further investigation is required to determine the utility of the material.



a b

Figure 6.2 L929 cells incubated with (a) positive cytotoxicity control sample and (b) negative cytotoxicity control sample over 24 hours.

6.3.2 Clotting Time

The initial event that occurs when blood contacts a foreign surface is the adsorption of plasma proteins, followed by a complex series of reactions that include the activation of blood-clotting enzymes and adhesion and activation of blood platelets and leukocytes, desorption and or further adsorption of proteins (5 ,6). When an abnormal situation comes, the cells in the blood trigger the blood-clotting cascade. The initiation of clotting cascade mainly depends upon the situation and foreign surface. Material mediated clotting initiation depends on the nature of the surface properties of the material. Lee and Neville reported the clotting times for various untreated plastics and indicated different clotting times for different base polymers (7). Slow clotting initiation means clotting time is more and fast clotting initiation indicates less clotting time. In the biocompatibility point of view a long clotting time indicates the material is suitable for blood contact applications.

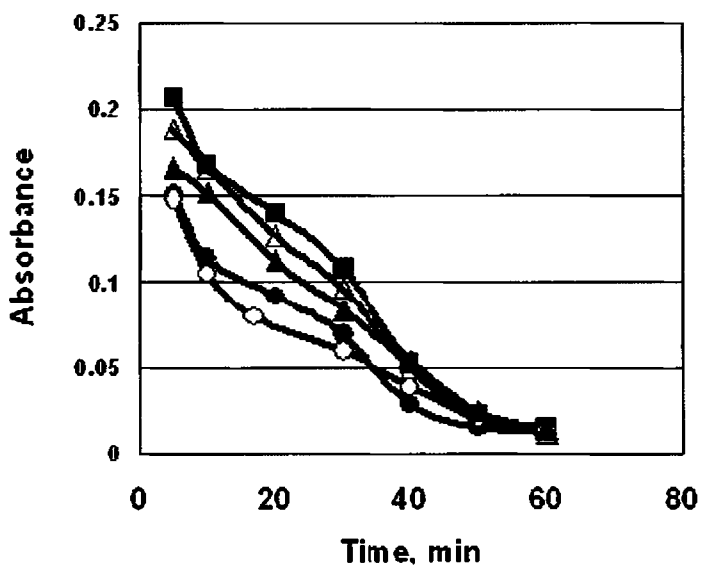


Figure 6.3 Comparison of clotting times of the samples (●) pPPVC, (■) EVA12, (○) mPO, (▲) AE1225 and (△) AE1250

In the whole blood clotting time test method a specific quantity (0.1ml) of blood was placed on the surface of the material. After a predetermined time the specimen was transferred into a beaker containing 50ml of water. The red blood cells that has not been trapped in the thrombus were haemolysed in the water and was measured at 540 nm in a uv-visible spectrophotometer. The optical density of the solution was monitered as a function of time and was plotted as shown in the 6.3. Conventionally, the time at which the optical density decreases to 0.1 is regarded as the clotting time.

The results show that blending of EVA with mPO increased the clotting time. It further shows that the clotting time increases with increase of EVA content. The modified samples, AE1225 and AE1250 show longer clotting time than pPVC, which in turn indicates that both these modified samples are better than PPVC from the biocompatibility point of view.

6.3.3 Cell Adhesion Studies

When blood contacts with an artificial surfaces, a series of events are initiated: rapid adsorption of a layer of plasma proteins at the interface, platelet adhesion to the protein layer and activation of the coagulation system to form thrombin and fibrin and culminate in thrombus formation (8,9). Platelets are anucleated fragments of megakaryocytes, which are formed in the extra sinusoidal space of the bone marrow. The typical shape of resting platelets is discoid (Figure 6.4a). Platelets are the smallest corpuscular components of human blood (diameter 2-4μm) - the physiological number varies from 150,000 to 300,000 mm3 blood. Despite their appearance on the face of it platelets are not cells, as they are not provided with a nucleus. Platelets circulate in the blood and their normal life span is 8-10 days and 1/3 of the platelet mass is transiently sequestered in the vascular pool of the spleen, exchanging freely with the systemic vascular pool. Platelets have important physiological role in maintaining vascular integrity and the arrest of bleeding by its interaction in association with coagulation factors with the component of vessel wall (10).

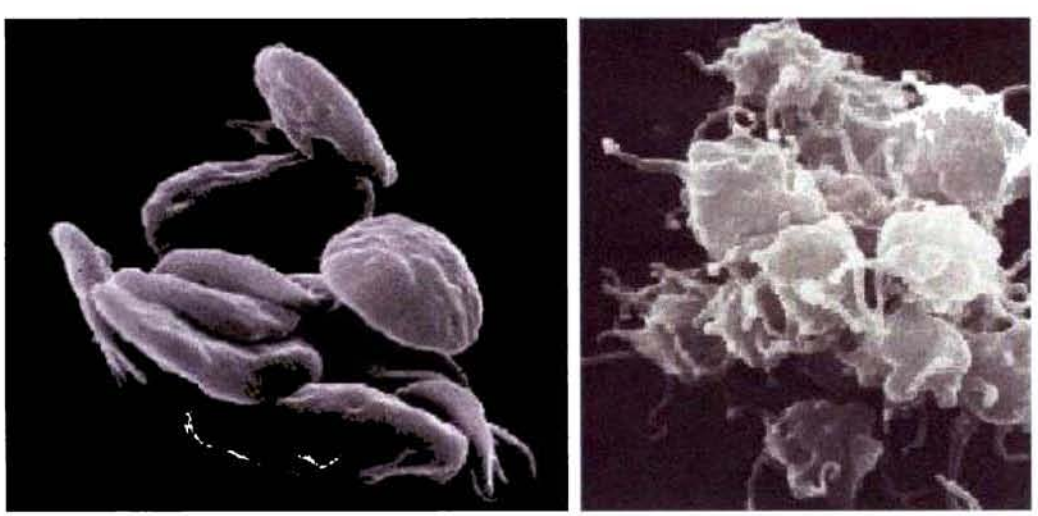


Figure 6.4: Scanning electron micrographs of platelets (a) and its activated form (b)

Platelets play a major role in determining the short-term thrombogenicity of artificial surfaces exposed to blood. When platelets come in contact with any artificial surface, they get activated and upon activation they undergo a shape change to a globular form with pseudopodia (Figure 6.4b). Platelet-material surface interactions are major determinant of thrombus when blood is exposed to artificial surfaces (11). Similarly, leukocytes are another type of cells found in the blood. The adhesion of these cells to theartificial surfaces also give rise to cell activation and the generation of complement and coagulation proteasis, which in turn may mediate thrombus formation and inflammatory reactions (12). So in the biocompatibility evaluation of materials to be used for blood contact application, the adhesion studies of platelets and leukocytes are very much important and essential.

The cell adhesion test was carried out as per the International Standard ISO10993-4: 2002 (13). Blood from human volunteer was collected into the anicoagulant, CPD. The test materials were

placed in polystyrene culture plates and immersed in phosphate buffered saline before they were exposed to blood. To each plate 1.5ml blood was added and a 0.5ml sample was collected immediately for cell count. Three samples were tested for each material. Three empty polystyrene culture dishes were exposed with blood as reference. The count reduction was analysed by detecting the counts in initial and 75min samples using Haematology Analyzer (Cobas Minos vet, Roche, France). Total consumption from the exposed blood as the percentage reduction is calculated for each sample. The total consumption means that the number of platelets or cells adhered to the artificial surface. Less count reduction means that less number of particles is adhered on the artificial surface and is more advantageous from the biocompatibility point of view.

The percentage platelet count reduction on the sample surfaces is shown in the figure 6.5. The results indicate that the platelet count reduction is more in the case of virgin m-PO (no modification) compared to PPVC. So it may be inferred that the virgin m-PO is less biocompatible than PPVC. A low level reduction of cells from the medium can be seen when a modified material comes in contact with it. This indirectly indicates that the modification of mPO with EVA reduces adhesion of cells onto the surfaceand this is a very encouraging result as far as the biocompatibility is concerned.

Similarly, the leukocyte-material interaction study as shown in figure 6.6 indicated that very less number of cells is adhered to the virgin and modified samples of mPO compared to PPVC. Since further leukocytes count reduction was not possible from the virgin material from the practical point of view, the assessment of the modification was not possible.

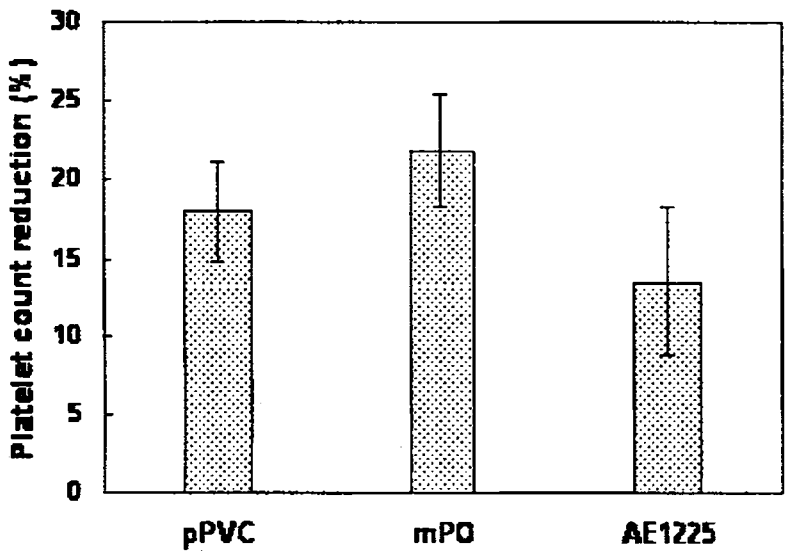


Figure 6.5 Percentage platelet count reduction on the sample surfaces

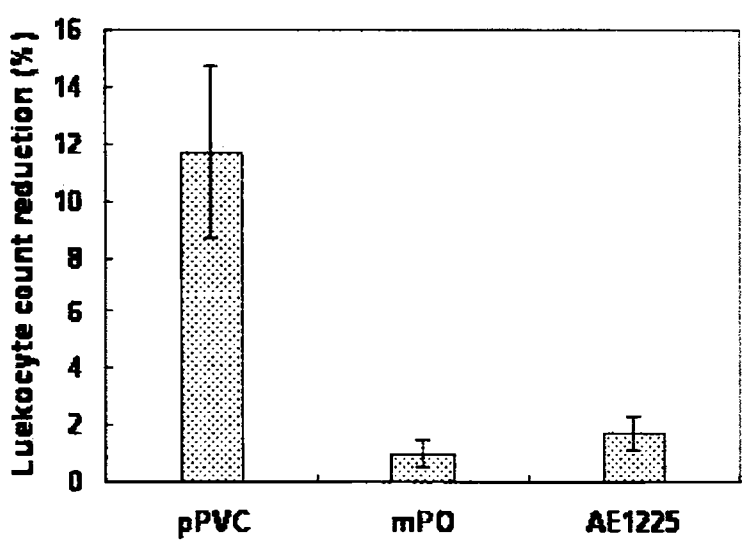


Figure 6.6 Percentage leucocyte count reduction on the sample surfaces

For the visual assessment of the platelet and leukocytes adhesion onto the artificial surface, 1.0 ml of the blood was exposed to the materials for 30 min under agitation at 75±5 rpm using an Environ shaker thermostated at 35±2^{o}C. The materials after lapse of the time period were rinsed thoroughly with poshphate buffered saline and

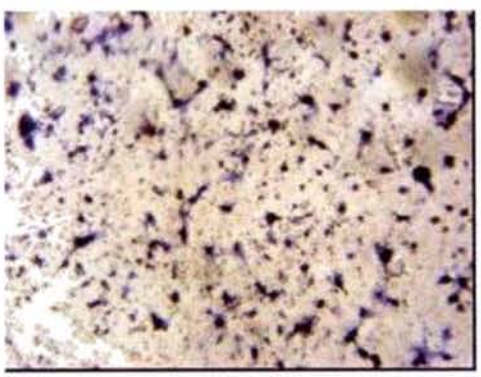


Figure 6.7 Adhered cell on pPVC sample surface

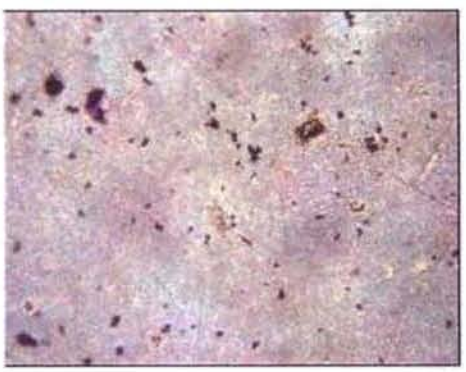


Figure 6.8 Adhered cell on m-PO sample surface

Figure 6.9 Adhered cell on modified m-PO sample (AE1225) surface

were fixed with 1% glutaraldehyde for 1 hour. They were then stained with May Grunwalds stain and viewed under light microscope to detect cell adhesion. A large number of platelets and leukocytes were seen adhered on the pPVC sample surface (figure 6.7). Adhesion of cells was very less in the case of virgin m-PO and light microscopic results indicate that the modification of m-PO improved the non-thrombogenicity of the material (figure 6.8 & figure 6.9)

6.3.4 Haemolysis

Red blood cells are extremely numerous in the blood stream and each has an average life expectancy of about four months. The destruction of red blood cells appears to be a major concern when plastic implants are to be in contact with flowing blood on a long-term basis. Plastic surfaces have been implicated in immediate and delayed haemolysis.

Comparison of percentage haemolysis of virgin mPO and its modified version (AE1225) was carried out with pPVC and the results are given in the figure 6.10. The results indicate that the percentage haemolysis is less for both the virgin mPO and the modified sample AE1225. It further shows that the modification mPO with EVA reduced the percentage haemolysis.

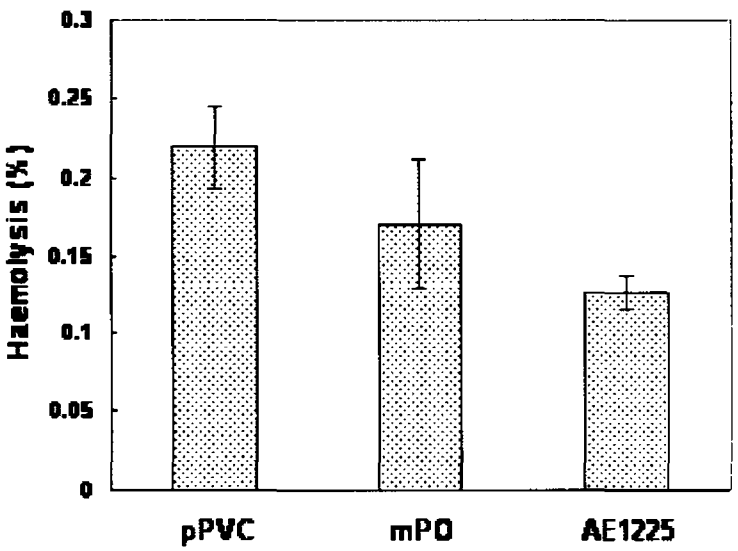


Figure 6.10 Comparison of perectage haemolysis of virgin mPO modified sample (AE1225) and pPVC.

6.4 Conclusion

The biological evaluation of the blend composition AE1225 was investigated based on the guidelines/protocol prepared by ISO-10993

Results of the cytotoxicity potential evaluation revealed that no cellular degeneration or malformation occurred to the L929 cell lines when exposed to the blend composition AE1225. The cell line P929 retained their original morphology even after being in contact with the samples either directly or by means of the extracts from the material, suggesting that the material is non-cytotoxic to the cell-lines used for the stduy.

The data on clotting time of the materials show that the modification of mPO with EVA increased the clotting time of the virgin polymer. On comparison of the clotting time of the blend AE1225 with PPVC the former showed a higher value indicating its potential candidature for the replacement of pPVC.

The reduced percentage reduction of the platelets and leucocytes counts in the material exposed blood samples indicated that the modification of mPO with EVA reduces cells loss from the blood, which in turn indicated less adhesion of the cells on the sample surface. This result was confirmed with the microscopic surface evaluation of the materials. Compared to pPVC the

virgin mPO and the blend AE1225 shows less adhesion of the cells which is a positive, and encouraging result in the cell adhesion study point of view.

The haemolysis test carried out gives an understanding about the material mediated blood cell lysis, especially the leucocytes. The results showed that the virgin mPO and the blend AE1225 were less haemolytic than PPVC. Also the modification of mPO with EVA reduced the haemolytic nature of the material.

The in vitro blood compatibility evaluation of the materials showthat the blend AE1225 exhibits better haemocompatibility and lower cell adhesion compared to that of plasticized PVC. Therefore the blend is a promising material for blood /blood component storage

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Chapter 7 Summary and Conclusions

7

SUMMARY AND CONCLUSIONS

The importance of developing or formulating a most suitable material in the success of a project cannot be overemphasized. Plasticised poly (vinyl chloride) (pPVC), the most important material in the biomedical field, is at the crossroads. Migration of plasticizer into the body fluids from pPVC during its service life and the environmental pollution after its service life has prompted the scientific community to look for other materials to replace pPVC. This study has been undertaken in this context.

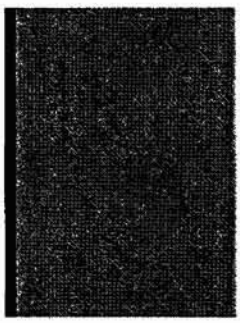
The first part of the study has been to reduce the plasticizer(DEHP) extraction and thus to make plasticized PVC less troublesome. For this end three polymeric plasticizers namely NBR, ENR and XNBR were tried to partially replace DEHP. PVC/NBR has been found to be the most promising material due to its good mechanical properties and reduced leaching of DEHP compared to PVC/ENR and PVC/XNBR. Further, PVC/NBR system was found to be nontoxic in the preliminary toxicity evaluation by in vitro cytotoxicity studies. But limitations of the system were the reduction in gas permeability and the increase in water vapour transmission rate. These limitations may affect the long-term storage of biological fluids especially the platelet concentrate in the system. However, PVC/NBR system is definitely superior to plasticized PVC for other applications like medical tubings where the permeability is not an important criterion.

The second part of the study has been to explore the possibility of completely replacing pPVC. For this, a novel polymer, metallocene based polyethylene (mPO), was chosen as the base material. As per the literature, this class of materials has superior mechanical properties and better transparency than conventional polymers due to its narrow molecular weight distribution and has been projected as a potential candidate in the biomedical field. Even though many applications have been identified for these materials, its use in the medical field is not seriously explored. One potential polymer from this group was selected for our studies based on the mechanical properties. The material was evaluated by measuring properties like mechanical

,water and gas permeabilities, transparency, preliminary toxicity, blood clotting time test etc. mPO was found to be superior to pPVC and EVA (the conventional materials used for medical applications) in mechanical properties and resistance to water permeability. But mPO was found to be inferior to pPVC and EVA in gas permeability as well as compatibility with blood. In order to overcome these limitations mPO was modified with EVA. The modified material was found to be superior to pPVC in mechanical properties, resistance to water permeability and swelling, transparency and had almost comparable contact angle and oxygen and carbon dioxide permeability characteristics. To find the effect of sterilization, the modified material was subjected to gamma radiation. The effect of gamma ray syterilization on the the blend was evaluated by comparing the technical properties before and after sterilization.

A mPO/EVA12 was found to be promising candidate for replacing pPVC and was subjected to the preliminary toxicity evaluation by in vitro cell culture cytotoxicity tests. The material mediated toxicity and the toxicity due to leachables were evaluated by the 'direct contact' and 'test on extract' test methods. The in vitro cell culture cytotoxicity studies using mouse fibroblasts cell line (L929) showed that the modified materials were non-cytotoxic to the L929 cell line. The modified material was further evaluated for its biological performance with blood. In vitro blood compatibility analysises like Haemolysis, Cell adhesion and Clotting time were performed according to the methods recommended as per ISO-10993 for blood-material interactions. The analysis for haemolysis showed that the modified material had low haemolytic potential compared to pPVC which indicates that the modified material causes less cell wall lysis or less damage to blood cells compared to pPVC. The clotting time test showed that the modification of mPO with EVA enhanced the dlotting time. The increase in clotting time of the modified sample, AE1225 makes it superior to pPVC. The cell adhesion test was carried out as per the International Standard ISO-10993-4: 2002 using the blood from human volunteers. The percentage platelet count reduction in the medium was analysed . A higher platelet count reduction in the medium of mPO indicated higher adhesion of platelets on to mPO surface. But the modification of mPO with EVA reduced the adhesion of platelets onto the surface. Also the modified sample showed a less platelet adhesion compared to pPVC. In the case of leukocytes, red blood cells, the adhesion was found to be less on to the virgin as well as the modified sample surfaces compared to pPVC. So this system is found to be superior to pPVC in biological analysis also.

The study shows that PVC/NBR and mPO/EVA blend are suitable for short term blood contact applications and the latter for long term application too. mPO/EVA system can be used for the complete replacement of pPVC.



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