THE EFFECT OF SURFACE PROPERTY TOWARDS BACTERIAL BIOFILM FORMATION

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UNIVERSITI SAINS MALAYSIA

2022

SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING

UNIVERSITI SAINS MALAYSIA

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by

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Dissertation submitted in partial fulfilment of the requirements for the degree of Bachelor of Engineering with Honours

(Materials Engineering)

Universiti Sains Malaysia

August 2022

DECLARATIONS

I hereby declare that I have conducted, compiled the researched work and written the dissertation entitled "**The Effect of Surface Property Towards Bacterial Biofilm Formation**", I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this for any other examining body or university.

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ACKNOWLEDGEMENT

Bismillahirrahmanirrahim. First and foremost, I would like to express my gratitude to Allah SWT for giving us opportunity and help us endlessly in finishing the final year project. I would like to express my gratitude to the Dean of School of Materials and Mineral Resources Engineering Universiti Sains Malaysia, Prof. Ir. Dr. Mariatti Jaafar and School of Materials and Mineral Resources Engineering (SMMRE) Universiti Sains Malaysia for providing necessary resources, facilities, and materials complete this project successfully.

Furthermore, I would like to express immeasurable and deepest gratitude to my supervisor Prof. Ir. Dr. Srimala Sreekantan, for her guidance, supervision, and continuous support while completing this project. Not to forget, I would like to express sincere gratitude to my co-supervisor, Dr. Rabiatul Basria for the invaluable guidance throughout this project that I have also been doing at Oncological and Radiological Sciences Cluster, Advanced Medical & Dental Institute (AMDI).

I would also like to thanks to the technicians and administrations of SMMRE, USM and AMDI, USM, for their support and assistance in making the study a success.

Besides, I would like to thank my family, friends and individuals who have contributed to my work directly or indirectly. Thank you, for your good deeds and enlighten all the time, and I pray that Allah would easy your journey. I am extending my heartfelt thanks to my parents for their endless support and prayers to complete this research work.

This research was supported by the Ministry of Higher Education through the Fundamental Research Grant Scheme (FRGS) (Grant No.: 203.PBAHAN.6071502).

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LIST OF ABBREVIATIONS

AFM	Atomic Force Microscopy
DEHA	Di(2-ethylhexyl) adipate
DEHT	Di(2-ethylhexyl) terephthalate
CAGR	Compound annual growth rate
EPS	Extracellular polysaccharides
EU	European Union
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infrared
PC	Platelets concentrate
PVC-DEHP	Polyvinyl chloride (di(2-ethylhexyl) phthalate)
RBC	Red blood cell
SBF	Simulated body fluid
SEBS/PP	Styrene-ethylene-butylene-styrene - polypropylene
SEM	Scanning Electron Microscope
TOTM	Tris(2-ethylhexyl) benzene-1,2,4-tricarboxylate
WCA	Water Contact Angle
USM	Universiti Sains Malaysia

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ABSTRAK

Beg darah PVC-DEHP komersial mempunyai beberapa kelemahan yang menjadikan filem SEBS/PP sebagai polimer alternatif untuk menggantikannya. Sebagai contoh, pengumpulan platelet dan pembekuan darah berlaku akibat larut lesap pemplastis DEHP ke dalam darah, sekali gus menjadikan keseluruhan beg darah mempunyai jangka hayat yang singkat selama 42 hari kerana bahaya jangkitan kuman. Oleh itu, tujuan penyelidikan ini adalah untuk menyiasat sifat permukaan filem SEBS/PP sebagai polimer alternatif untuk menggantikan beg darah PVC semasa dan bagaimana ia mempengaruhi pembentukan biofilm bakteria. Pertama, filem SEBS/PP dihasilkan dengan menggunakan acuan filem tiupan dan menjalani beberapa pencirian seperti FTIR, SEM, WCA dan AFM. Kemudian, kajian sifat antibakteria telah dijalankan dengan mengikut garis panduan CLSI MO2-A11 dan diikuti dengan penilaian pembentukan biofilm bakteria. Dua strain bakteria diperoleh daripada American Type Culture Collection (ATCC); E. coli ATCC 22922 dan S. aureus ATCC 25923 dan dikultur semalaman. Selain itu, bioaktiviti SEBS/PP disiasat dengan menggunakan Cecair Badan Simulasi (SBF). Filem SEBS/PP menunjukkan mempunyai sifat permukaan yang boleh mengakibatkan pengurangan lekatan bakteria kerana ia terdiri daripada sudut sentuhan permukaan yang lebih tinggi daripada filem PVC-DEHP. Permukaan ia juga mampu mengurangkan sedikit lekatan bakteria apabila bersentuhan dengan bakteria dengan sifat kebolehbasahan yang berbeza.

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ABSTRACT

The commercial PVC-DEHP blood bag has several drawbacks which make SEBS/PP film as an alternative polymer to replace it. For example, platelets aggregation and blood clotting occurred due to the leaching of DEHP plasticizer into the blood, thus making the whole blood bags have a short 42-day shelf life due to the danger of bacterial infection. Therefore, the purpose of this research is to investigate the surface properties of SEBS/PP film as an alternative polymer to substitute the current PVC blood bags and how it affects the formation of bacterial biofilm. Firstly, the SEBS/PP film was produced by using blown film molding and undergo several characterizations such as Fourier Transform Infrared (FTIR) Spectroscopy, Scanning Electron Microscopy (SEM), Water Contact Analysis and Atomic Force Microscopy (AFM). Then, antibacterial properties study was carried out by following the CLSI MO2- A11 guideline and followed by bacterial biofilm formation assessment. Two strains of bacteria were obtained from American Type Culture Collection (ATCC); *E. coli* ATCC 22922 and *S. aureus* ATCC 25923 and cultured overnight. Moreover, the bioactivity of SEBS/PP is investigated by using Simulated Body Fluid (SBF). SEBS/PP film showed to have the surface properties that can result in reduction of bacterial adherence as it consists of a higher surface contact angle then PVC-DEHP film. SEBS/PP's surface is also able to slightly reduce the bacteria adhesion when in contact with bacteria with different wettability properties.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Polyvinyl chloride (PVC)-DEHP (di(2-ethylhexyl)phthalate) blood bags have been widely utilized for blood transfusions all over the world for years. DEHP is used as a plasticizer for polyvinyl chloride (PVC) in order to increase the flexibility of the polymer as well as reducing its brittleness. Therefore, most of the PVC medical equipment contain DEHP as a plasticizer (Bider et al., 2020).

Nevertheless, the leaching of DEHP has been discovered over the years which affects the erythrocyte membranes, thus causing issues in neonates and patients receiving hemolysis treatment in hospitals (Erythropel et al., 2014). The International Agency for Research on Cancer (IARC) has concluded that there is sufficient evidence of DEHP's carcinogenicity in experimental animals. As a result, DEHP has been categorized as a Group 2B carcinogen that can cause cancer in humans. DEHP that is integrated into the inner and membrane fractions of erythrocytes cause a stabilizing impact on the erythrocyte membrane by lowering osmotic fragility as well as hemolysis (Serrano et al., 2016). Due to its lipophilic properties, DEHP migrates into stored blood components, implying that the presence and amounts of plasma lipids will promote the migration which is also known to be dependent on temperature (Bider et al., 2020; Lee et al., 2018). However, recent epidemiological research on the impact of DEHP exposure on testosterone production, breast cancer, and other issues were considered either inconclusive or inconsistent (European Commission, 2017).

In spite of that, PVC-DEHP blood bags are still intensely developed and used over the years due to their excellent physical properties and affordability which are

favorable with the current economic situation. The transfusion medical community has been unable to eliminate DEHP from storage bags and tubes for more than 20 years, thus resulting in DEHP toxicity in recipients and donors (Cuenca et al., 2020).

Realizing the DEHP toxicity, it is timely to arrest this problem by replacing the current PVC-DEHP blood bags with an alternative polymer, SEBS/PP blend that comes with good mechanical and biochemical properties (Hassan, 2021). This alternative will completely eliminate the toxicity of using DEHP as a plasticizer in PVC blood bags.

1.2 Problem Statement

a) Toxicity effect of DEHP that may cause to the blood contaminants

The major drawback of currently PVC-DEHP blood bags is the leaching of DEHP into the blood components whereas it can cause various health problems such as breast cancers, osteoporosis, hepatomegaly, peroxisome proliferation, brain development issues, decreased male fertility, as well as reduction in body weight. Moreover, the EU chemical agency classifies DEHP as an endocrine disruptor, which means it may affect how normal hormones functioning (Hervey, 2019). Therefore, it has long been wanted to replace DEHP in blood bags with a comparable non-toxic plasticizer, but not at the expense of blood component quality (Scientific Committee on Emerging and Newly Identified Health Risks, 2015; van der Meer et al., 2014). Thus, an alternative plasticizer has been suggested to avoid the problems encountered with DEHP, which are di(2-ethylhexyl) terephthalate (DEHT) that have shown the greatest potential by providing inferior RBC quality in previous studies (Graminske et al., 2018;

Larsson et al., 2020; Serrano et al., 2016). The study shows that PVC blood bags plasticized with DEHT maintain sufficient red blood cell quality throughout a 49-day period of storage and do not significantly change the platelet lesion profile or the degradation of coagulation components in plasma. Bags made of PVC-DEHT maintain the same high level of physical and functional quality as bags made of DEHP (Larsson et al., 2020). However, this method still cannot achieve the target to produce a blood bag with zero composition of plasticizer in it. For that reason, a blood bag formulation without the need of plasticizer is introduced by employing a combination of SEBS/PP for the blood bag formulation to avoid the problem of plasticizer leaching into the blood.

b) Bacterial infection of the blood.

Bacterial biofilm formation is known as communities of microorganisms that are attached to a surface and plays a significant role in the persistence of bacterial infections. This becomes a problem when the biofilm formation contributes to undetected bacterial contamination of stored blood products in a blood bag. Because the bacteria in the biofilm are adherent rather than planktonic, it will be difficult to detect them using conventional methods. In September 2014, a patient who was suffering from chronic myelogenous leukaemia was transfused with two 5-day-old buffy coat platelet (PLT) pools. Later in the same day, he returned to emergency with a low-grade fever, thus bacteremic and died on the next day. After microbiology and molecular testing, the testing revealed that this S. epidermidis isolate infects platelet concentrates (PC) with a biofilm-positive phenotype which somehow gives a false-negative BacT/ALERT screening result (Kou et al., 2015).

This BacT/Alert system is a continuously monitored blood culture system for detecting the presence of viable bacteria in the circulating blood. Meanwhile in 2018, the U.S Food and Drug Administration has reported that seven deaths were attributable to contamination which six were caused by bacterial contamination with 2 cases involving *Staphylococcus aureus* and one was caused by parasite contamination from *Babesia microti*. Over the past five years, Staphylococcus aureus has continued to be the most common infectious agent found in apheresis platelets (FDA, 2018, 2019). Therefore, certain pathogen reduction technologies have been developed to eliminate the necessity for bacterial screening prior to transfusion. The INTERCEPT Blood System (Cerus Corp.) is one of them which has completed Phase III clinical trials. This system employs amotosalen HCl, which binds to nucleic acids on ultraviolet light (UVA) irradiation, thus restricting the bacterial growth in platelets. Although this technique is effective against a wide range of bacteria, it has failed to destroy *Bacillus cereus* spores and to completely eliminate high loads of *Pseudomonas aeruginosa* and *Enterobacter cloacae* (Diallo et al., 2020). In order to avoid transfusion of contaminated platelets, this study will also be focused on antibacterial potential of the polymer film as well as the formation of bacterial biofilm on the SEBS/PP film as it becomes an alternative polymer blend to replace the current PVC blood bag.

1.3 Research Objectives

The objectives of this study are

1. to investigate the surface properties of the SEBS/PP film prepared by blown film moulding method as an alternative to plasticizer free PVC bag.

2. to examine the antibacterial potential and biofilm formation of the SEBS/PP polymer film.

3. to evaluate the bioactivity study of the SEBS/PP using Simulated Body Fluid (SBF) solution.

1.4 Scope of Study

The research scopes involved 5 main stages. Firstly, the fabrication of SEBS/PP film was conducted using blown film moulding. In the second stage, the surface characterization methods are conducted starting by evaluation of surface morphology under FE-SEM. The sample is first washed and sterilised with 70% of ethanol and distilled water. For cross sectional observation, the sample is immersed in liquid nitrogen for cryofracture. Then, FTIR analysis is done for determining the chemical functional groups that exist in the SEBS/PP blend and PVC blood bag. In order to study surface topography and roughness, both SEBS/PP and PVC-DEHP are observed under the Atomic Force Microscope (AFM) by operating at the contact mode. The results were analysed by using NanoNavi and Gwyddion softwares. Last for this stage, the surface polarity of the sample is evaluated by observing the water contact angle of distilled water on their surface. Then in stage three, antibacterial potential study of polymer film is investigated by following the standards known as Disk Diffusion Method (CLSI MO2-A11) which involves two strains of bacteria, *E.coli* and *S. aureus*. This is followed by stage four, whereby assessment of bacterial biofilm formation which involved crystal violet assay and qualitative evaluation bacterial interaction by SEM was elucidated using the same bacteria strains as before. The bacteria biofilm is grown in a 96-well plate with different incubation time; 24h, 5 days and 7 days. After the incubation time, the sample is taken out from the well and

proceeds to fixation in order to be observed under the SEM, while the 96-well plate continues with the crystal violet assay. In stage 5, bioactivity study of the polymer blend by using Simulated Body Fluid (SBF) solution was evaluated by observing its weight and the pH of the buffer throughout 7 days of incubation.

1.5 Thesis Outline

This thesis consists of five chapters. Chapter 1 elaborates a brief introduction, problem statement, objectives and scope of the research. Chapter 2 a comprehensive review on the blood bag market overview, blood bag system, requirements for blood bag, processing steps of PVC blood bag, currents blood bag; pros and cons, blood compatible polymer, bacterial biofilm formation and surface characteristic influencing bacterial adhesion. Chapter 3 details the experiment procedures that are used in this study. This includes the experimental design, production of polymer blend film, surface characterization techniques, antibacterial potential study, bacterial biofilm formation assessment and bioactivity of the sample. This chapter also covers a brief explanation on the characterization equipment, their operation principles and sample preparation. Chapter 4 describes the experimental results and comprehensive discussion on the effect of surface property towards bacterial biofilm formation. Finally, Chapter 5 is devoted to the conclusion of this research work and suggestions for future work and development.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter will narrate the basic concept of surface properties of the polymer blend and the bacterial biofilm formation that are important for the blood bag application. This study could provide benefit advancement in the blood bag application as this study removed undesirable toxicity on the current blood bag. Topics that have been discussed here are blood bag market overview, blood bag and its components, requirements of blood bag, processing steps of blood bag, current PVC blood bag, blood compatible polymers, bacterial biofilm formation and surface characteristic influencing bacterial adhesion.

2.2 Blood bag market overview.

A sterile, clear plastic device used for the transfer, storage, collection, and transfusion of blood and its components is referred to as a disposable blood bag. A blood bag is made of biocompatible PVC materials and additives to keep blood and its components safe until they are transfused or used again. The blood bags market is estimated to gain market growth in the forecast period of 2020 to 2030. The market for disposable blood bags was predicted to be worth $221.1 million in 2020 and is anticipated to expand to $357.2 million by 2030, with a compound annual growth rate (CAGR) of 4.9% between 2021 and 2030 (Talekar et al., 2022).

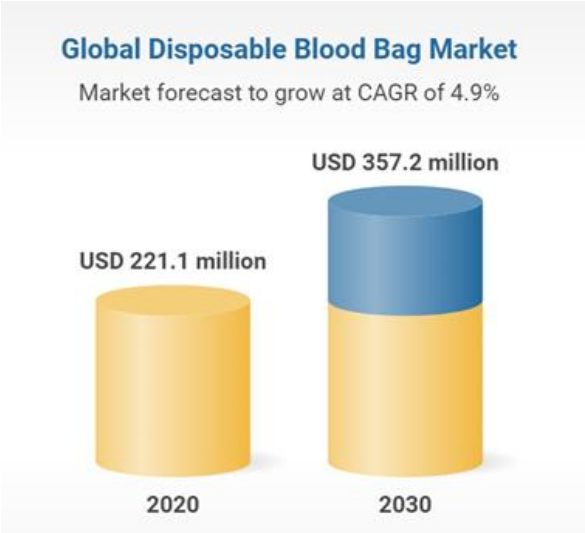


Figure 2.1 Disposable blood bag market overview. (Talekar et al., 2022)

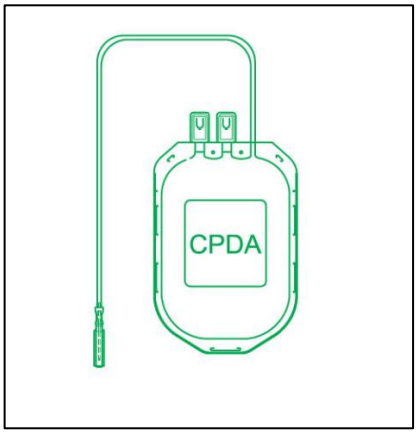
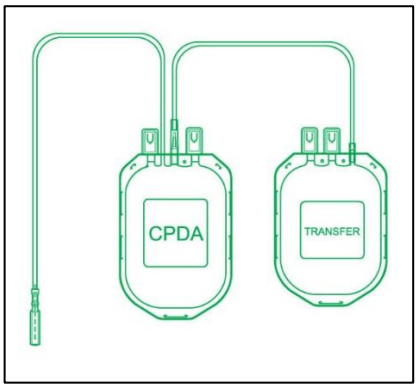
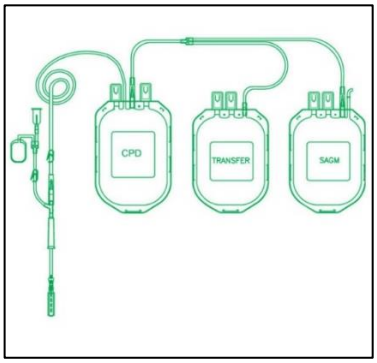
The blood bag market is growing due to an increase in blood transfusion operations and an increase in the incidence of injuries and accidents around the world. According to a report published by the World Health Organization (WHO) in 2020, roughly 1.35 million people die each year as a result of traffic accidents. Non-fatal injuries affect 20-50 million individuals worldwide, with many of them resulting in disability. Despite having 60% of the world's vehicles, low- and middle-income countries account for 93% of all road deaths. Individuals from lower socioeconomic backgrounds are more likely to be involved in road traffic crashes, even in high-income countries. As a result, rising traffic-related crashes result in more demand for blood bags, which may show a significant market growth over the forecast period.

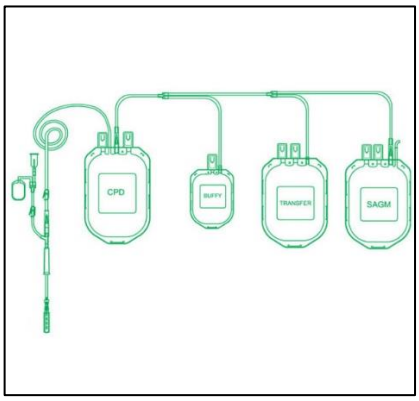
2.3 Blood bag system, types and uses.

The current PVC blood bag consists of blood donor bag, labels, needle with needle cover, break off valve, tubing and outlet port pouch. As secondary packaging, they are wrapped in laminated polyester/aluminium/polyethylene packaging. It helps to lower the moisture content, ensures sterility from the outside, and preserves the bag from damages. There are 4 types of blood bags which are single, double, triple and

quadruple blood bag. Table 2.1 showed all types of blood bag and its components as well as its functions (Terumo Penpol, 2021).

Table 2.1 Types of blood bags (Terumo Penpol, 2021).

Types	Components & Uses
Single Blood Bag 	▪ For whole blood collection. ▪ The bag contains an anticoagulant solution which is known as **CPDA** solution. **Citrate** - acts as anticoagulant by chelating calcium. **Phosphate** - lower acidity and have a higher concentration of 2,3 DPG and red cell phosphate. **Dextrose** - needed for metabolism of stored red blood cells. **Adenine** - improves the viability of red blood cells.
Double Blood Bag 	▪ For whole blood collection. ▪ Primary bag contains CDPA solution for a whole blood collection and store the red cell concentrate component after centrifugation. ▪ An additional empty bag called a transfer or satellite bag which will store plasma component after centrifugation.
Triple Blood Bag 	▪ For whole blood collection and separation of 3 different blood components (RBC, plasma and platelets. ▪ Only the addition of an additional transfer bag distinguishes a triple bag from a double bag. ▪ The primary bag contains CPD and one satellite bag contains SAGM which is

	known as a blood bag additive that extends the shelf life of RBC from 35 to 42 days.
Quadruple Blood Bag  	▪ For whole blood collection and separation of 3 different blood components (RBC, plasma and platelets) through the buffy method. ▪ The primary bag contains CPD and 3 satellite bags; 1 satellite bag is 100ml capacity to prepare platelets through the buffy coat technique, 1 satellite bag contains SAGM which is known as a blood bag additive that extends the shelf life of RBC from 35 to 42 days. ▪ One satellite bag is suitable for 5 days of platelet preservation.

2.4 Requirements for blood bag.

Based on ISO 3826-1:2019, the plastics container must be practically colorless, flexible, sterile, nonpyrogenic, biologically safe, and non-frangible under conditions of use. It must be compatible with the contents under usual storage conditions. The plastic container must comply with the requirements for terminal sterilization and must not become tacky while being sterilized and stored for the duration of its shelf life at temperatures below 40 °C.

Throughout the duration of its shelf life, the plastic container must be physically, chemically, and biologically stable with respect to its contents and must prevent the penetration of microorganisms. Blood and blood components, as well as any other chemicals that may be released from the plastic container by the anticoagulant and/or preservative solution contained within, through chemical interaction or physical breakdown, must not exceed the permitted limits.

In Table 2.2, some of the crucial requirements for a blood bag are summarized, and the details are elaborated upon in the following section.

Table 2.2 Blood bag requirements.

Requirements	Remarks	Reference
Mechanical Requirement	Tensile strength: >40 MPa Young's Modulus: <0.1GPa	(Sojiphan et al., 2004)
Thermal Requirement	Able to endure temperatures as low as -80 °C (freezing) & as high as 120 °C (sterilization) Melting point: >120 °C	(ISO 3826- 1:2019, 2019)
Safety and Compatibility Requirement	Must not react with any gases or liquid.	(ISO 3826- 1:2019, 2019)
Manufacturing Requirement and Others	Must be lightweight (around 350 g)	(Sojiphan et al., 2004)
Cost	Low in cost (RM23/unit)	(Terumo Penpol, 2021)

2.4.1 Physical Requirements (*ISO 3826-3 Plastics Collapsible Containers for Human Blood and Blood Components-Part 3: Blood Bag Systems with Integrated Features*, 2006)

2.4.1(a) Conditions of manufacture

The plastics container must be manufactured, assembled, and stored under conditions that are clean and hygienic at all times. Every practical measure must be taken to limit the possibility of cross contamination with microbes or foreign substances.

2.4.1(b) Sterilization

The plastic container must have undergone steam sterilization or another approved technique of sterilization. The method of sterilization employed shall not

negatively affect the materials or contents, nor shall it result in any loosening of joints and degradation of welds in the plastics material, nor shall it result in any material alteration of the plastics container's shape.

2.4.1(c) Transparency

When looking through the plastic container, the suspension's opalescence must be seen in comparison to an identical plastic container filled with water.

2.4.1(d) Coloration

The material of the sterilized plastics container must not be colored in a way that would make it difficult to judge the blood's color.

2.4.1(e) Thermal stability

According to ISO 3696, the plastic container must withstand being filled with water to half of its nominal capacity, slow freezing to -80 °C for 24 hours, subsequent immersion in water at (37 ± 2) °C for 60 min, and then returning to a temperature of (23 ± 2) °C. Plastic containers intended for blast-freezing (shock-freezing) or irradiation must be certified for those particular applications. In order to prevent direct contact between the plastics container and the refrigerant solution if one is used, the plastics container may be wrapped in a protective bag.

2.4.1(f) Water vapour transmission

Without an overpackage, the plastics container must be filled with water to its nominal capacity in accordance with ISO 3696, sealed, and labelled "ready for use." The plastic container must subsequently be able to withstand 42 days of storage at (4 ± 2) °C without losing a mass fraction of more than 2% of the solution's water. Specific oxygen and carbon dioxide gas exchange rates may be necessary for the storage of some blood components, such as platelet concentrates.

2.4.1(g) Resistance to leakage

The plastics container must not leak during centrifuging at 5000 g for 10 min at 37 °C while being filled to nominal capacity with water as specified in ISO 3696 and sealed. The plastics container is then compressed between two plates for 10 minutes at (23 ± 2) °C and a gauge pressure of 50 kPa. Visual inspection should reveal no leaking.

When a plastic container is filled with an anticoagulant solution, such as an ACD solution or another solution with a same pH, leakage can be found by pushing the plastic container against blue litmus paper and watching for the appearance of pink spots on the paper. The same technique can be applied with the appropriate indicator for solutions with different pH levels. Alternative techniques are allowed as long as they have at least the same level of sensitivity.

2.4.1(h) Particulate contamination

Plastic containers must be produced with the least amount of particle contamination allowed. It is possible to employ the limits and test protocols listed in pharmacopoeias, such as those described for parenteral solutions in the European Pharmacopoeia.

2.4.2 Chemical Requirements

2.4.2(a) Requirements for the raw container or sheeting

The sheeting must correspond to the specifications given in the applicable pharmacopoeias.

2.4.2(b) Requirements for the test fluid

When the proper tests are done on the extract obtained in accordance with Annex A (List of chemical test), the limitations mentioned in Table 2.3 shall not be exceeded.

Table 2.3 Chemical requirements for blood bag.

Characteristics	**Maximum permissible value**	**Test method in**
Oxidizable constituents	1,5 ml	A.4.1
Ammonia	0,8 mg/l	A.4.2
Chloride ions (Cl–)	4 mg/l	A.4.3
Metals: Ba, Cr, Cu, Pb Sn, Cd Al	 For each metal: 1 mg/l For each metal: 0,1 mg/l 0,05 mg/l	A.4.4.1
Heavy metals	2 mg/l	A.4.4.2
Acidity or alkalinity	0,4 ml sodium hydroxide solution, c(NaOH) = 0,01 mol/l or 0,8 ml hydrochloric acid, c(HCl) = 0,01 mol/l	A.4.5
Residue on evaporation	5 mg or 50 mg/l	A.4.6
Opalescence	Slightly opalescent, but not more pronounced than that of reference suspension	A.4.7
Coloration	No coloration	A.4.8
UV absorbance	In the range of 230 nm to 360 nm 0,25 for plastics containers with a nominal capacity ≤ 100 ml and 0,2 for plastics containers with a nominal capacity > 100 ml	A.4.9

Human blood and blood components should only be stored in plastic containers made from carefully selected materials to reduce the danger of chemical compounds leaching into the product. The toxicity of the ingredients employed and the biological compatibility of the plastic container with the product must both be taken into consideration.

2.4.3 Biological Requirements

The plastic container must not compromise the therapeutic efficacy of blood and blood components and must not release any compounds that could cause excessive toxic, cytotoxic, bacteriostatic, bactericidal, pyrogenic, or haemolytic reactions. The ISO 10993 series lists the common biological safety tests.

2.4.3(a) Impermeability for microorganisms

The plastics container shall be impermeable to microorganisms.

2.4.3(b) Compatibility

The plastic containers must not discharge any compounds into the anticoagulant/preservative solution and/or blood, or blood components in amounts that can cause pyrogenic, poisonous, or hemolytic effects.

2.5 Processing steps of PVC blood bag.

The following section outlines the several steps involved in making a blood bag:

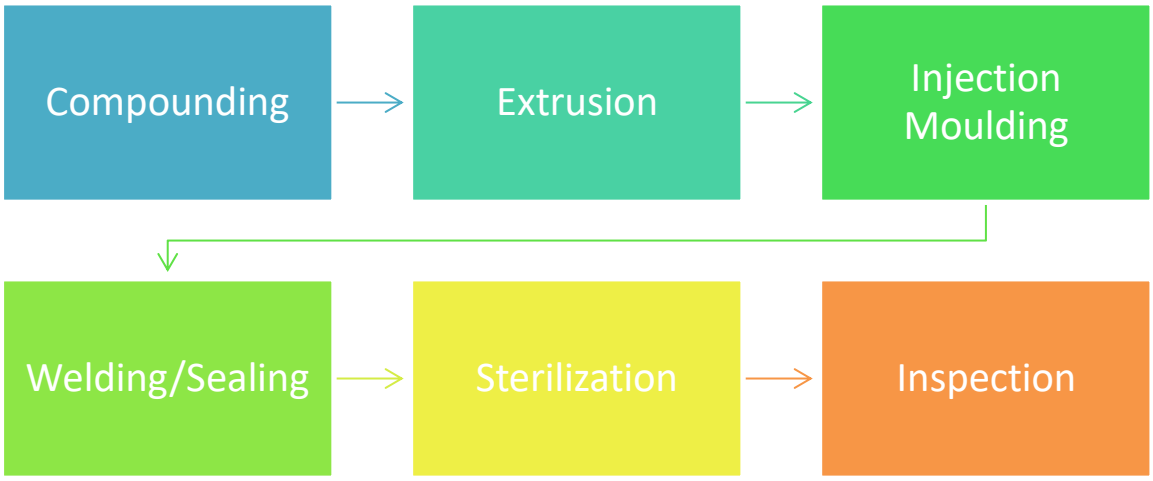


Figure 2.2 Processing steps of PVC blood bag fabrication (TechSci Research, 2021).

2.5.1 Compounding

This is an essential process in the production of PVC-DEHP blood bags which involves combining plasticizers, additives, and polymers. In order to obtain a uniform blend of the polymers with additional additives like plasticizers and stabilizers, this technique is typically carried out while it is molten.

2.5.2 Extrusion

The compounded PVC is then extruded through a die to transform the plasticized material into sheets. The extruded sheet is slit, then it is cut to desired size and sent to the welding department. Extrusion method is also used to create the donor and transfer tubing which made of PVC composites. The tubes are sent to the welding area after being printed and cut to the proper length in line.

2.5.3 Injection Moulding

Components like the transfusion port, needle cover, clamp, and so forth are made using injection moulding. Before usage, the components are ultrasonically cleaned and dried in an oven.

2.5.4 Welding/Sealing

By applying a high-frequency welding process, the blood bags are manufactured. PVC sheets that are in the right size are positioned between the electrodes where a high frequency at a high voltage is subjected. The PVC heats up very quickly during the heating process, resulting in sealing between the electrodes. The blood bag system is then put together by welding the welded bag, the donor and transfer tubes, transfusion ports, and other parts in the proper position, thus followed by a trimming.

2.5.5 Sterilization

"Air-over-steam" sterilization, also known as the autoclaving, is used to sterilize the PVC blood bags infusing steam into a pressure chamber at a temperature range of 120 - 130 °C with 7000 Pa for 30 minutes, which it is enough time to eliminate any germs present on the blood bag. It is advisable to sterilize blood bags in autoclaves that can carry out pure saturated steam programs, with full vacuum and a steam-heated jacket. Autoclaves are more adaptable for the specific treatment of blood bags as well as for any other materials or products due to the small additional expense (Mascherpa, 2015).

2.5.6 Inspection

This process involves a complete inspection to get rid of any visible flaws such leaks, debris, and other visible defects. Thereafter, the inspected bags are packed in laminated polyester/aluminium/polyethylene packaging (Terumo Penpol, 2021).

2.6 Current blood bags – PVC- DEHP blood bags – Issues and Disadvantages

The current blood bags that have been commercialized over the years are the Polyvinyl chloride (PVC)-DEHP(di(2-ethylhexyl) phthalate) blood bags. These blood bags are still intensely developed due to their excellent physical properties and affordability with the current economic situation. Each year in Europe, approximately $3x10^4$ tonnes of plasticized PVC is used for medical applications such as IV and blood bags and infusion tubing, enteral and parenteral nutrition feeding bags, cardiopulmonary bypass, and extracorporeal membrane oxygenation system tubing (Radke et al., 2018). DEHP is also shown to have beneficial effects to the blood bag application as it reduces hemolysis susceptibility of the red blood cells stored and

minimizes the formation of both echinocytes and microparticles (Melzak et al., 2018). DEHP plasticizer also has been chosen due to its ability to withstand steam sterilization while retaining its elasticity and flexibility (Sarker, 2020).

However, the use of DEHP as a plasticizer becomes a significant problem as when leaching has occurred during the storage of blood. According to several studies, DEHP has been leached and found to have harmful effects in newborns, children, and patients who require regular blood transfusions. The high exposure of DEHP to humans can lead to cancer as it is considered a human carcinogen by The International Agency for Research on Cancer (IARC). Leaching of DEHP also can cause blood contamination, thus blood clotting. Therefore, Gulliksson et al. (2017) has introduced a new blood bag manufactured of polyolefin to avoid any potentially migrating substance into the blood. The research was shown to give encouraging results as red blood cells could be stored for 28 days, but the method was unable to prevent the increase of hemolysis.

2.7 Blood compatible polymer.

Due to their versatility, biocompatibility, and ease of production, polymers are frequently utilized in the field of biomedical science. They also exhibit a wide spectrum of mechanical, chemical, and thermal properties. Table 2.4 shows the list of polymer blends that have been used in the past in the field of medical science.

Table 2.4 List of polymer blends and their respective medical application.

Polymer Blends	Applications	References
Polyhydroxy butyrate (PHB) & Thermoplastic polyurethane	Tissue engineering	(Song, Chang and Naguib, 2015)
(PCL) and (PU)	Permanent implants	(Cooke and Whittington, 2016)
PP/SEBS	Medical Packaging	(Sandholzer, Bernreitner and Klimke, 2016)
Quaternized chitosan/polyvinyl alcohol/sodium carboxymethylcellulose	Wound dressing	(Hu, Qiang and Wang, 2017)
PP/EPDM blend	Urinary catheter	(Balaji et al., 2017)
(Hydroxyethyl)methacrylate	Dental adhesives	(Rodrigues et al., 2018)
PP & PE	Medical device, laboratory syringe	(Gopanna et al., 2019)
PHB functionalized with piperazine	Anti-cancerous activities toward in vivo Ehrlich ascitic carcinoma	(Singh et al., 2019)
Blend of Parylene	Cardiovascular stent	(Golda-Cepa et al., 2020)
Gelatine/PVA	Hard tissue regeneration	(Wang et al., 2020)
PDMS/polyvinylpyrrolidone (PVP)/fumed silica (FS)	Bio-medical application	(Zarrintaj et al., 2020)

Since the focus of the research is alternative materials for PVC-DEHP blood bags, there have already been a number of attempts to produce PVC- and DEHP-free blood bags. A list of these attempts is shown in Table 2.5 below.

Table 2.5 List of previous research attempts.

Attempts	Compound	Critical Effect	Shelf Life (days)	References
DEHP platicizer-free	DEHA	Developmental toxicity resulting in skeletal variation.	28	(Van Vliet et al., 2011)
	TOTM	Lung changes observed in rats from inhalation.	21	(Testai et al., 2016)
	DEHT	All PCs were briefly in contact with DEHP when passing through the IPP system.	49	(Larsson et al., 2020)
PVC blood Bag	DEHP	Reported damage to neonates and young kids.	42	(Sampson and De Korte, 2011)
PVC-free polymers	Polyolefin based PVC free blood bag.	Resulted in high haemolysis levels within three weeks, surpassing the European maximum capacity of 0.8%.	28	(Gulliksson et al., 2017)

Table 2.5 lists all of the PVC-DEHP free blood bags that have been tried in the past. None of them were successful in stopping the haemolysis level from the blood bags from decreasing. The numerous allegations that PVC-DEHP has negative effects on newborns and environmental health inspired the launch of the PVC FREE BLOOD BAG project in 2011, which was completed in 2017 and produced PVC-Free blood bags. Alternative polymers for blood bags are mentioned in the project report and the list is shown in Table 2.6.

Table 2.6 List of identified polymers as an alternative material for blood bags.

Identified Polymer	**Temperature Range (°C)**	**Tensile Strength (MPa)**	**References**
Ethylene Vinyl Acetate (EVA)	110	24.68	(Stark and Jaunich, 2011)
Ethylene-Propylene Copolymer	160	21.00	(Rungswang et al., 2013)
Polyethylene (PE)	135	31.02	(Jeong et al., 2018)
Polyethylene terephthalate	260	60.00	(Zander, Gillan and Lambeth, 2018)
Polypropylene (PP)	160	40.00	(Baruah et al., 2018)
Styrene-ethylene-butadiene-styrene (SEBS)	190	20.00	(L. Liu et al., 2019)

Based on Table 2.6, PP and SEBS are selected as SEBS/PP blend has been utilized in the past for medical applications. It is anticipated that the mixing of PP with SEBS will result in new PP/SEBS thermoplastic elastomeric materials with a broader range of properties, including improved elastomeric behavior and processability. The flexibility and elasticity of the produced material can be customized by adjusting the thermoplastic elastomer content in the blends (Kiehle, Roth, and Wießner, 2019). To function as a blood bag material, polymers must possess a number of characteristics, including mechanical properties, thermal characteristics, and sealability. The list of polymers and their properties are therefore provided in Table 2.7.

Table 2.7 List of polymers and their properties for blood bag.

Polymers	Mechanical Properties (MPa)	Thermal Properties (°C)	Sealable	References
SEBS	15 - 20	-25 to 185	Yes	(Lefakane et al., 2015)
PP	20 - 50	-20 to 175	Yes	(Remananet al., 2019)
LDPE	4 - 15	-130 to 100	No	(Ganesan et al., 2021)
EVA	7 - 17	-10 to 95	No	(Junior et al., 2021)
PU	1 - 14	-20 to 90	No	(Xu et al., 2021)

From Table 2.7, SEBS and PP also have been chosen as both polymers possessed excellent thermal and mechanical properties as well as both are sealable. The market also tends to favour styrene ethylene butylene styrene (SEBS) TPE block copolymers due to their superior compatibility with polyolefins (polypropylene, polyethylene), excellent weathering resistance, excellent thermal stability, and the ability to modify the chain in order to produce materials with low hardness, high elasticity, and high optical properties.

Despite frequent claims that products made of vinyl are hazardous to human health or the environment, PVC uses are still expanded. One drawback of switching from PVC containers to alternative materials is the early absence of scale economies that have favoured PVC in the IV-container product line (Chung, 2019). This impediment has delayed adoption of innovative products since consumers are typically hesitant to pay a higher price for alternative materials in today's cost-conscious market.