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Plasticization of PVC membranes with eugenol for Biomedical Applications

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ABSTRACT

PVC polymer has been taken the attention of the scientists in early decay as blood containers instead of glass containers. To increase the stability and flexibility of the PVC, several compounds such as Di-ethylhexyl phthalate (DEHP) and ethylene oxide were applied. Some recent reports recorded the risks of these compounds toward public health. In the present study, Antimicrobial PVC films containing different amounts of eugenol as a plasticizer were prepared using traditional casting method. The physical and mechanical properties of PVC membranes e.g. surface wettability were investigated. The increase of eugenol content demonstrated an increase in surface hydrophilicity and elongation to break the film. Thermal analysis exhibited a decrease of polymer thermal stability by increasing eugenol concentration. However, the antibacterial activities against six different bacterial strains; three Gram positive: *Staphylococcus aureus*, *Streptococcus pyogenes* and *Bacillus cereus* as well as, three Gram negative: *Pseudomonas aeruginosa*, *Salmonella* sp. and *Escherichia coli* were promoted by addition of eugenol. Although the natural source of eugenol, the bio-evaluation of plasticized membranes showed an increase in hemolysis percent (%) and thrombus weight. It can be concluded that the addition of eugenol to PVC needs to further studies for applying in blood bags.

INTRODUCTION

Starting from the 17th century, transfers of blood from healthy volunteer to patients was established as a medical treatment for some diseases and during a surgical operation.[1] Until 1950, glass bottles were the most standard containers for blood transfusions and storage. Several problems were associated with using these old fashion vessels related to sterilizations, breakability, and the presence of air bubbles surround valves and during a blood transfusion [2, 3]. All of these reasons driving scientists to develop a new generation of flexible bags based on polymeric materials. Polyvinylchloride (PVC) plays an essential role in modern design bags, due to its inertness, durability, and resistance to heat/cold, chemicals, abrasion, and kinking [4].
In common with virtually all plastics, PVC is composed of a polymerized organic substance, in this case, polymerized vinyl chloride, together with one

or more additives that modify the characteristics of the polymer to optimize its suitability for a given application or process. PVC is used in some situations with minimal additives, in which case it is a hard rigid material and suitable for some construction purposes. Most usually, however, the best performance can be achieved when the material is made softer and more flexible. For this purpose, an additive described as a plasticizer is used, and the resulting plasticized, or soft, PVC finds widespread applications. A plasticizer can be defined as a material incorporated into the plastic to increase its flexibility [5]. A plasticizer used in plastic to confer softness to a polymer has to be a low molecular weight substance that effectively acts as a molecular lubricant. Di-ethyl hexyl phthalate (DEHP) is the most common plasticizer for PVC-based medical devices such as tubings, intravenous bags, blood containers, and catheters. From 30–40 percent of blood bags use DEHP as plasticizer [6]. DEHP can easily elute from PVC products into solutions that contact with the plastic and the migrated DEHP is directly and/or indirectly introduced into the human body [7-9]. By 1967, Guess reported that DEHP leached from the plastic blood bags into plasma [10]. Later, Jaeger and Rubin detected DEHP that present at levels of 50–70 mg/l [11]. Leakage of DEHP depends on some factors, including the lipids con¬tent in the blood, the storage time and the temperature [12]. DEHP is classified as a reproductive toxin [13].

Self-disinfected PVC membranes to be used in biomedical take attention of scientists in last decay. Several approaches were done to goal this goal. Including grafting of PVC surfaces with biocidal molecules to prevent bacterial growth on the surface. Indeed, antibacterial PVC surfaces were successfully prepared by grafting of Heparin [14], Chitosan [15, 16], PEG [17], silver nitrate [18], aromatic thiol compounds [19].

Today the modern studies awarded to increase antimicrobial and haemocompatibility of the native polymer besides improving mechanical and physical properties of the polymer itself without adding any hazard materials.

The aim of the work is to plasticize PVC with eugenol that considered the main component of clove oil instead of synthetic plasticizer DEHP to be used in blood bag applications.

MATERIALS AND METHODS

Materials: Polyvinyl chloride (M.W. ~48000)**,** fine powder belami fine chemicals, (India). Tetrahydrofuran (THF) (Purity 99.9%) sisco research laboratories (India). Eugenol (purity 99%, M.W.

164.2) Sigma-Aldrich (Germany), Yeast Extract; bacteriological grade, Biobasic Inc. (Canda). Peptone powder; bacteriological grade, Biobasic Inc.(Canda). Sodium chloride (Purity 99.5%, M.W. 58.44) International co for Supp&Med. Industries, (Egypt). Monosodium phosphate (Purity 98 %, M.W. 119.98, Sigma-Aldrich, Germany). Disodium phosphate (Purity 98%, M.W. 117.98, Sigma-Aldrich, Germany). Sodium chloride (Purity 99.5%, M.W.58.44, El-Nasr company, Egypt).

Bacteria: Five bacterial strains were used for evaluating the antibacterial activity of plasticized membranes. These were included three Gram-positive (*S. aureus*, *S. pyogenes* and *B. cereus*) and three Gram-negative strains (*E. coli, P. aeruginosa and Salmonella* sp.) and. The strains were refreshed through inoculating in Luria Britani (LB) broth (peptone 1%, yeast extract 0.5%, NaCl 1% and pH 7±0.2) and were incubated overnight at 37°C and 150 rpm in a rotary shaker.

Methods
Preparation of PVC membranes: The membranes were prepared by using traditional casting method. 1 g of PVC was dissolved in 25 ml THF at room temperature. A definite amount of eugenol was added to the solution under stirring to obtain a homogenous solution. On a clean glass petri dish, the solution was cast at room temperature and was left for 48 hrs to ensure complete solvent evaporation. Once the membrane was dried and separated from the Petri dish, it was rinsed with 50 ml of distilled water. The wet membranes were spread out and allowed to dry for 24 hrs at room temperature. Seven different plasticized PVC membranes with eugenol were studied and coded as sample PVC 0, PVC-EG1, PVC-EG2, PVC-EG3, PVC-EG4, PVC-EG5and PVC-EG6 containing different amounts of eugenol (0, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5 ml) respectively.

Fourier Transform Infrared Spectrophotometer (FT-IR): The structures of plasticized PVC membranes were investigated by carrying FT-IR spectroscopic analyses using Fourier Transform Infrared Spectrophotometer (Shimadzu FTIR - 8400 S, Japan) following the method which was described by Tamer *et al*., [20].

Thermal gravimetric analysis (TGA): Thermal analyses of plasticized PVC membranes were carried out using Thermal Gravimetric Analyzer (Shimadzu TGA −50, Japan).

Mechanical properties: Mechanical properties were performed to characterize plasticized PVC

membranes for confirming the reproducibility of the membrane formation technique. These properties include the membrane thickness, and maximum stress and strain to failure. A method for testing the tensile properties of the film was adopted according to ASTM D- 882 standards for testing tensile properties of paper and paper broad using a constant rate of elongation apparatus. The instrument that used to test these properties was an AG-1S, Shimadzu, Japan. Membrane thickness measurements were measured with an electronic digital micrometer.

Contact angle measurements: Static water contact angle measurements were performed at room temperature using (advanced Gonimeter model 500-F1) in a sessile drop configuration (using ultrapure water as the liquid), coupled with a video camera and image analysis software. At least, ten droplet images were obtained for each film.

Antibacterial activity
A. **Broth evaluation method:** The antibacterial activities of plasticized PVC membranes were tested as the method which was described by Skyttä and Mattila [21]. In this experiment, three bacterial strains were used (*S. aureus, S. pyogenes and Shigella* sp.). Briefly, the bacteria were inoculated in a Luria- Bertani medium (LB medium) (1% peptone, 0.5% yeast extract, and 1% NaCl) and were incubated at 37ºC for 24 hrs in a rotary shaker. The obtained bacterial suspensions were diluted with the same media solution till 100 times. 0.1 ml of diluted bacteria suspensions were cultured in 10 ml broth media, which contains a piece of membranes (5 cm X 1 cm). The inoculated media were incubated at 37ºC for 24 hrs with shaking. The optical density of cultures was measured using spectrophotometer at 620 nm.

B. **Agar diffusion method:** Agar-well diffusion method was applied for screening the antimicrobial activities of plasticized PVC membranes against *E. coli*, *P. aeruginosa*, *Salmonella* sp., *S. aureus* and *B. cereus* as described by the previous method [22, 23]. Briefly, 0.5 ml overnight cultures of the indicator microorganisms were swabbed on LB agar medium (1% peptone, 0.5% yeast extract, 1% NaCl and 1.5% agar). Discs of PVC membranes were placed on the agar surface, and the plates were left in the refrigerator at 4°C for 2 hrs to allow the diffusion of materials into the agar. The plates were incubated at 37°C for 24 hrs then; they were investigated and photographed via gel documentation system.

Evaluation of haemocompatibility: The hemolysis tests were performed as described in American Society for Testing and Materials (ASTM) (ASTM F 756-00, 2000) [24, 25]. Anticoagulated blood was used for this purpose. This sample was prepared by adding 1 ml of anticoagulant acid citrate dextrose solution (ACD) to 9 ml of fresh blood. Before performing the tests, Samples (1 cm^2) were placed in polypropylene test tubes and 7 ml of phosphate buffer saline solution (PBS) pH 7.0 were added. After 72 hrs of incubation at 37°C, the PBS was removed, and 1 ml ACD blood was added to each sample and maintained at 37°C for 3 hrs. Positive and negative controls were prepared by adding the same amount of ACD blood to 7 ml of water and PBS respectively. Each tube was gently inverted twice each 30 min to maintain contact of the blood with the material. After incubation, each fluid was transferred to a suitable tube and centrifuged at 2000 rpm for 15 min. The hemoglobin released by hemolysis was measured by the optical densities (OD) of the supernatants at 540 nm using a spectrophotometer (Pharmacia Biotech Ultrospec 2000).
The percentage of hemolysis was calculated as follows equation:

$$\text{Haemolysis (\%)} = [(OD_{\text{sample}} - OD_{\text{negative control}})/(OD_{\text{positive control}} - OD_{\text{negative control}})]$$

According to ASTM F 756-00 (2000) materials can be classified into three different categories according to their haemolytic index (%): materials with percentages of haemolysis over 5% are considered haemolytic; while the ones with haemolytic index between 5% and 2% are classified as slightly haemolytic. Finally, when the material presents a hemolysis percentage below 2%, it is considered as a non-haemolytic material.

Thrombogenicity: Evaluation of thrombus formation on polymeric surfaces was carried out using a gravimetric method [26]. Anticoagulated blood was used for this purpose as mentioned above. Before performing the tests, samples were immersed in PBS at a constant temperature of 37°C. After 48 hrs of incubation, the PBS was removed, and the ACD blood was put in contact with the surface of the membranes and also to an empty Petri dish, which acted as a positive control. Blood clotting tests were initiated by adding 20 µl of a 10 M calcium chloride solution and were stopped after 45 min by the addition of 5 ml of water. Resultant clots were fixed with 5 ml of a 36% formaldehyde solution and were then dried with tissue paper and finally weighted. Each test was carried out in three determinations, and the mean values were calculated.

RESULTS AND DISCUSSION

In the current study, plasticization of PVC membranes with active biocidal molecules were conducted for dual functions; improvement the mechanical properties of polymer and exhibit self-antibacterial properties to PVC membranes.

FT-IR analysis: The vibration modes and wave numbers exhibited by PVC are C–H stretching mode observed at 2947 cm^{-1}, CH$_2$ deformation mode at 1340 cm^{-1}, C–H rocking mode at 1247 cm^{-1}, trans C–H wagging mode at 958 cm^{-1}, C–Cl stretching mode at 856 cm^{-1}, and cis C–H wagging mode at 619 cm^{-1} as shown in Fig. 1. Plasticization of PVC membranes showing new bands related to eugenol start to seen in the chart by increasing the content of plasticizer inside membranes; OH Phenolic at 3520 cm^{-1}, =C-H stretching at 3060 cm^{-1} and aromatic –C=C-stretching at 1514 and 1616 cm^{-1}.
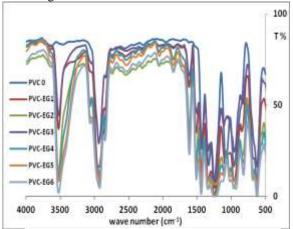

Figure 1: FT-IR of PVC and PVC plasticized membranes with different amounts of eugenol.

Thermal gravimetric analysis (TGA): Figure 2 illustrates thermal decomposition of PVC membranes with different content of eugenol. It is clear that two distinct stages of degradation were exhibited in the decomposition of PVC. The first decomposition started from 247°C to 305°C with a peak decomposition temperature at 280°C.

This stage of degradation was attributed to autocatalytic dehydrochlorination reaction (zipper elimination) with the subsequent formation of conjugated double bonds [27-30]. After the loss of the first HCl molecule, the subsequent unsaturated structure formed in a PVC chain is an allylic chlorine structure. However, this allylic chlorine (Scheme 2) stimulates the next loss of an HCl molecule, and the repeated process leads to the chain or zip dehydrochlorination [31].

The second decomposition started from 450°C, which presumably corresponded to the degradation of the resulting unsaturated hydrocarbons in the dehydrochlorination of PVC. The addition of eugenol showing new peak starting from 100°C to next depression that attributed to evaporate eugenol from polymer chains [31].
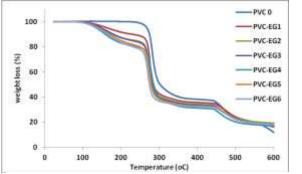

Figure 2: Thermal degradation of and PVC plasticized membranes with different amounts of eugenol.

Mechanical properties: Mechanical properties of PVC membranes plasticized with different amounts of eugenol were determined from critical breaking point of stretching. Maximum stress σ_{max} (Nm^{-2}) was evaluated as the ratio of the stretching force divided by the cross-sectional area of broken membrane piece. The maximum strain λ_{max} was measured as the elongation ratio of the initial length of the test piece. The results showed that there is a general decrease in maximum stress by increasing eugenol content as in Table 1. In the same way, Elongation percent was increased due to the lubricant action of eugenol between PVC chains.

Table 1: Mechanical parameters of PVC membranes plasticized with different amounts of eugenol.

Sample	Max_Stress σ_{max} (Nm^{-2})	Max_Strain λ_{max} (%)
PVC 0	50.1645	1.4295
PVC-EG1	63.3224	1.4295
PVC-EG2	34.0712	1.427
PVC-EG3	24.72	47.71
PVC-EG4	21.439	55.497
PVC-EG5	20.7078	56.493
PVC-EG6	17.2086	65.202

Contact angle: Water contact angle of different PVC membranes was summarized as in Table 2. Table 2 illustrates the decrease of contact angle from θ =64° to be more acute angles in the presence of eugenol. These results show an increase of wettability of PVC

membranes by increase eugenol contents of it. Surface wettability (generally indicated to hydrophobicity/ hydrophilicity) is a useful parameter for measuring the biological response to an implanted material. Wettability affects protein adsorption, platelet adhesion/activation, blood coagulation and cell and bacterial adhesion [32–37]. However, observations regarding the effects of surface wettability on protein adhesion have not always been consistent. In generally hydrophobic surfaces are considered enable to adsorb proteins than hydrophilic surfaces because of the strong hydrophobic interactions occurring at these surfaces, in direct contrast to the repulsive solvation forces arising from strongly bound water at the hydrophilic surface [38-40].

Table 2: Contact angle of water on PVC membranes plasticized with different amounts of eugenol.

Sample	θR	θL	Mean
PVC 0	63.05	65.3	64.175
PVC-EG1	68.05	72.84	70.445
PVC-EG2	59.04	60.56	59.8
PVC-EG3	58.79	61.18	59.985
PVC-EG4	59.12	57.5	58.31
PVC-EG5	56.81	57.86	57.335
PVC-EG6	50.44	49.84	50.14

Bio evaluation

Antibacterial evaluation: Antibacterial evaluation of PVC membranes was carried out against both grams positive and negative bacteria using different methods. Figure 3 shows an increase in membranes activity against tested bacteria (i.e.; *S. aureus*, *S. pyogenes* and *Shigella* sp.) by increase eugenol content. These results confirmed by Agar-well diffusion method as shown in Figure 4. The activities of the prepared membranes against *P. aeruginosa* make these results is highly promising because this strain is a common pathogenic strains in different hospitals and has different resistant mechanisms versus most antibiotics [41, 42].

Haemocompatibility: Several essential requirements must be taken into consideration during preparation and qualification of medical devices, especially blood contact materials. Blood compatibility is recognized to play critical parameter during evaluation of wound dressing membranes. The value of Haemolysis is taken as a mentor test. Figure 5 illustrates haemolysis percent of the prepared membranes. Haemolysis is regarded as an especially significant screening test.
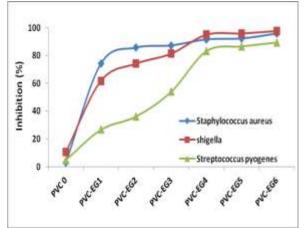

Figure 3: Antibacterial activity of PVC membranes plasticized with different amounts of eugenol.
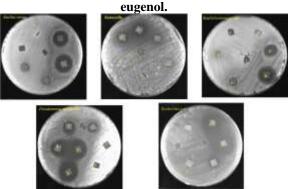

Figure 4: Antibacterial activity of PVC membranes plasticized with different amounts of eugenol using agar well diffusion method.

Once it provides quantification of small levels of plasma hemoglobin, which may not be measurable under *in vivo* conditions. As reported in the literature (ISO 10993-4(1999)), it is not possible to define a universal level of acceptable or unacceptable amounts of haemolysis. Although blood compatible materials should be non-haemolytic, several medical devices cause haemolysis in practice. This means that when such haemolytic effect takes place, it is important to make sure that clinical benefits overcome these risks and that the values of haemolysis are within acceptable limits. According to ASTM F 756-00 (2000), materials can be classified into three different categories according to their haemolytic index (haemolysis %): materials with percentages of haemolysis over than 5% are considered haemolytic; while the ones with haemolysis index between 5% and 2% are classified as slightly haemolytic. Finally, when material presents a haemolysis percentage below 2%, it is considered as a non-haemolytic material. Figure 5 shows a disastrous increase in haemolysis percent of blood contact with plasticized PVC.
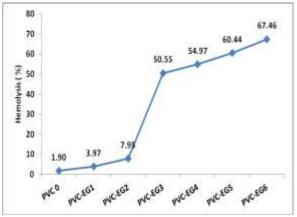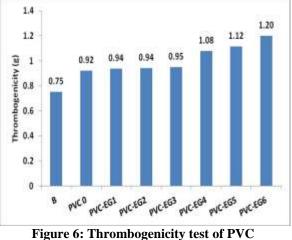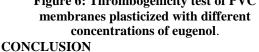
Figure 5: Haemocompatibility of PVC membranes plasticized with different amounts of eugenol.

Thrombogenicity: As the membrane is designed to be used topically in contact with blood, it is important to evaluate its tissue and blood compatibility. Furthermore, the thrombogenic character is a desirable property in membranes. Figure 6 shows the weights of blood clots obtained on thrombogenicity test. It was observed that clot formation was higher in membranes than in the control so; the polymers are classified as thrombogenic [27]. This characteristic is directly related to the hydrophilicity of the materials. When material placed in contact with a hydrophobic surface, proteins adsorb to it in a strong and irreversible way, while at hydrophilic surfaces proteins adsorb weakly and reversibly [43]. This relation between hydrophilicity and thrombosis was confirmed by the higher value of thrombus weight that was formed when blood contacted with PVC membranes, and that will increase by eugenol percent as a direct result of an increase in hydrophobicity.

Finally, the prepared membranes should be applied on animal model for more investigations [44].

Figure 6: Thrombogenicity test of PVC membranes plasticized with different concentrations of eugenol.

CONCLUSION

New PVC membranes plasticized with eugenol were prepared and characterized. Results showed dose improvement in membranes physical and mechanical properties. Additionally, Bio-evaluation study showed increasing of antibacterial activity against both Gram positive and Gram negative bacteria with increasing of eugenol concentrations. But at the same time, there are evident increases in blood hemolysis and thrombus weight. This study will be subjected to further analysis and investigations to obtain a good PVC membrane with potent features to apply in blood bags manufacture.

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