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Ionic polyurethane materials for enhanced platelets adhesion and biocompatibility

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Abstract: *Polyurethane ionomers (PUI) are employing as one of the prominent materials in the field of biomedical applications. PUI materials have been synthesized from the reaction of poly(ethylene glycol) [PEG] with diisocyanate reagent followed by the addition of dihydroxyl benzoic acid (DHBA) salt by two-step polymerization process. PUI material generated from NCO terminated polyurethane prepolymers bonded with ionomer fragment exhibit bio and haemocompatibility. All of these PUI films were characterized by FTIR and FT-NMR for structural identifications. Study of molecular weights and size analysis of PUI obtained were determined by Gel permeation chromatography and particle size analysis. Essentially, haemocompatibile nature of these PUI have been studied by thrombogenicity, haemolysis and platelet adhesion test. As such polyurethane linked with ionomer fragment exhibits better platelets adherence to soft or biological tissue.*
Keywords: *Bioadhesive, haemocompatibility, platelet adhesion, polyurethane ionomers, thrombogenicity*

I. Introduction

Many approaches have been developed to advance the technical proficiency of surgeons to accomplish a prompt and active control of wound closure, which is a critical factor in any surgical procedure. An instantaneous and satisfactory wound closure can reduce the span of patient inside operation theatre, under anaesthesia. Wound closure techniques are evolved from early development of suturing materials to comprise resources that include synthetic sutures, absorbables, staples, tapes, and adhesive materials. Till date the use of sutures has been the ubiquitous method [1] and possible alternative of sutures for wound closure [2] includes clip, staples can cause inflammation and wound infection. Materials such as synthetic and natural bioadhesives are alternative resolution for this problem [3]. In this context, attention is required to develop new synthetic bioadhesives rather than natural bioadhesives as some of them, reveal toxicity, and tissue bonding. Bioadhesion refers to adhesive [4, 5] interaction between biological tissue with natural or synthetic polymers, elucidated by adhesion theories. The rapid healing with aid of polyurethane (PU) film enabled with high elasticity and non-toxic property [6]. PUI from poly(oxytetramethylene glycol) [PTMG] as a precursor is reported via prepolymer method [7]. Our attempt to study bioadhesive interaction between polymers as bioadhesive with soft tissues prompted us to cast PUI film reaction from polyethylene glycol (PEG), isocyanates and DHBA salt by adopting standard procedures [8]. PU film formulation has been carried out by two main classes of catalyst, organometallics and tertiary amine based catalyst. PUI as promising biomaterials is accounted for its property and applications [9].

1.1 Polyurethane Ionomer (PUI)

Polyurethane based ionomer is an important category of ionomer, ion containing segmented polyurethanes [10]. Segmented polyurethanes are linear copolymers exists in the form [A-B]n, where block A consists of a relatively long and flexible soft segment and the block B is polar hard segment [10]. Especially, PEG affords soft segment and the hard segment arises from diisocyanate reagent as well as low molecular weight diol as a chain extender. Segmented polyurethane and ion containing segmented polyurethane differs by hard microdomains. In case of non-ionic polyurethanes, microphase separation occurs due to microdomains arising from hydrogen bonds among hard and soft segments [11, 12]. Materials of segmented polyurethanes containing ionic groups do generate additional electrostatic interactions in the region of hard segments. Also with respect to the existence of major weight fraction of the soft segments, hard microdomains are distributed among the continuous phase of soft segments, forming a three dimensional lattice [13]. Polyurethane ionomers are copolymers containing in the range of 2-15 mol % of ionic contents with desired physical properties like rubbery modulus and melt viscosity with respect to ionic interactions [13- 15]. The ionomers like cationomer, anionomer or zwitter ionomer proves its significant role [16]. Polyurethane based ionomers have been chosen as candidates for bioadhesives [17] due to its biocompatibility [18, 19]. We explore the preparation of polyurethane ionomer (PUI) by isocyanates prepolymer technique in order to demonstrate its biocompatibility.

II. Experimental Section

2.1 Materials

Poly(ethylene glycol) [PEG] average M_n 1000, hydroxyl benzoic acid (DHBA), isophorone diisocyanate [IPDI], tolylene-2,4-diisocyanate [TDI], 1,6-diisocyanatohexane [HMDI], 4,4'-methylenebis(cyclohexyl isocyanate) [H_{12}MDI], 4,4'-methylenebis(phenyl isocyanate) [MDI], Phosphate buffered saline (PBS) were procured from Aldrich, USA and were used as received. Dibutyltin dilaurate (DBTDL) was procured from Fluka Chemical Co., USA and was used without further purification. N, N'-dimethylformamide DMF (extrapure) was purchased from Sisco Research Laboratories, Mumbai. Ethyl methyl ketone (MEK) purchased from Ranbaxy Laboratory limited, Mumbai was used after purification.

2.2 Methods

2.2.1 Synthesis of Polyurethane Ionomers

10 g (0.01 mmol) of polyethylene glycol (PEG) was taken in a three necked flask and dissolved in MEK. A dropping funnel charged with diisocyanate 0.02 mmol, in MEK and added drop wise to the reaction flask. The reaction was continued for 5 h at 95 °C and then the reaction temperature was reduced to 50 °C. The chain extender, hydroquinone sulfonic acid (0.01 mmol) was added drop wise along with DBTDL catalyst (0.001 wt. %). Again the reaction mixture was raised to 90 ° C, maintained for another 30 min. The final polymer solution was casted on a mould [7, 20].

III. Characterization of Polyurethane Ionomers

3.1. Fourier Transform Infrared Spectroscopy (FTIR)

Nicolet impact 400 FTIR instrument was used to collect data on PUI products formulated from PEG. The IR spectra were collected by KBr pellet method and all the samples were recorded at a resolution of 4 cm^{-1} with 100 scans. Each time, a background spectrum was collected prior to record data for all PUI samples. The bands identified at various absorption frequencies infer the presence of various functional groups.

3.2. Fourier Transform Nuclear Magnetic Resonance (FT-NMR)

High-resolution ^1H & ^{13}C-NMR spectra were recorded using a Brucker MSL 300 P, 300 MHz FT-NMR spectrophotometer. Deuterated dimethyl sulfoxide (DMSO-d_6) was used as a solvent for recording NMR spectra. The proton spectra were recorded using broad band inverse probe where the inner coil is protons and outer coil for X nuclei. Solvent suppression was applied in some cases where the solvent signal is very strong compared to the sample signals. ^{13}C NMR was recorded in dual ($^{13}C/^1H$) probe where the inner coil is for ^{13}C nuclei and the outer coil is for protons. The decoupling of protons was done using Waltz-16 sequence. The spatial parameters like number of scans, time domain data points were adjusted depending on the nature of the sample and the relaxation parameters like T_1 and T_2.

3.3. Gel Permeation chromatography (GPC)

The number average molecular weight (Mn), weight average molecular weight (Mw) and polydispersity (Đ) of the synthesized polyurethane ionomers were determined by gel permeation chromatography using Waters unit interfaced with NEC (IBM AT Compatible) computer. Waters 515 HPLC pump columns (Ultrastyragel 103Å, 104 Å, 105 Å in series) coupled with a Waters 410 differential refractometer was used. This instrument was set at 40 ºC before starting the analysis. In GPC, a porous material is used as the stationary phase and a solvent as a mobile phase. The stationary phase used is a swollen gel of polstyrene and the mobile phase used is HPLC grade DMF. The flow rate of the solvent was maintained as 1 mL/min.

3.4. Haemocompatibility

Haemocompatibility is an essential feature to find out the compatibility of a material in contact with blood. Attempts that determine haemocompatibility often occur at the molecular level. Haemocompatibility can influence inflammatory processes. The major category of haemocompatibility evaluated in vitro by blood interactions with material is listed in Table 1.

Table 1 List of Haemolytic Grade

S. No	Haemolytic index (%)	Haemolytic grade
1	0-2	Non-haemolytic
2	2-5	Slightly haemolytic
3	> 5	Haemolytic

3.5. Haemolysis

ASTM F756-00 standard method was adopted to perform haemolysis tests. PUI film samples: (i) (PUI from TDI), (ii) (PUI from HMDI) and (iii) (PUI from IPDI) of each 21 cm^2 was treated with 7 ml of PBS and incubated at 37 °C for 72 h. After incubation, the PBS from individual film was removed and stored separately

after centrifugation at 2000 rpm for 15 min. The PBS untreated film samples were kept as control. To each of the untreated (3 Nos) and treated (3 Nos) films and the PBS extracts (3 Nos), added 1 ml diluted anticoagulated blood. All the nine samples were incubated at 37 $^{\circ}$C for 3 h, with intermittent gentle shaking at 30 min intervals. A positive control was prepared by adding 1 ml of anticoagulated blood with 7 ml water; and negative control was prepared adding 1 ml anticoagulated blood with 7 ml of PBS. Optical density (OD) values of supernatant fluid containing haemolytic product was measured spectrophotometerically at 540 nm. The percentage of haemolysis was calculated as described in the following equation.

$$\%\,\text{Haemolysis} = \frac{OD_{test} - OD_{negativecontrol}}{OD_{positivecontrol} - OD_{negativecontrol}} \quad \text{---------- (1)}$$

3.6. Thrombogenicity

PUI film samples of 1 cm^2 area was taken and placed in dry petri-dish. Blood was drawn from a healthy adult and a drop was added immediately to all the samples, before the blood sample undergo coagulation. Different period of incubation was scheduled for each sample, ranging from 10 to 50 minutes, and maintained accordingly. At the end of incubation, 25 ml of water was added to stop the clotting process of blood. The necessary precaution was taken to avoid disturbance on film or with clotted blood. After 5 minutes optical density of the fluid was measured in UV-vis spectrophotometer at 540 nm. A plot of OD values Vs. time drawn to study the thrombogenic property.

3.7. Platelet Adhesion Test

PUI films of 1.5 cm X 1.5 cm were incubated in PBS (0.1 M, pH 7.4) for 1 h. Blood sample of healthy human adult was anticoagulated with acid citrate dextrose (ACD) and platelet rich plasma (PRP) was isolated by centrifugation at 2500 rpm for 5 min. All the three PUI sample films were incubated in flat position on small petri-dishes submerged with PRP and left for 1 h at 37 $^{\circ}$C. After incubation, films were washed gently with PBS repeatedly to remove the non-adhering platelets. PUI film samples were then washed with distilled water, stained with leishman stain and examined in an optical microscope before being photographed.

IV. Results and Discussion

4.1. FT-IR Data

Analysis of FTIR data is substantial to identify the products [21]. Infrared spectra data for four samples of segmented polyurethane ionomer have been shown in Figs. 1-4. Stretching vibration of bonded and free -NH group was detected in the range of 3420-3330 cm^{-1}. The band appears in the range of 2980-2960 cm^{-1} supports the stretching vibration of C-H bond. Spectra data shows no absorption in the range of 2250-2270 cm^{-1}, which indicates the completion of polymerization reaction and absence of free -NCO groups. Intense band identified around 1720-1660 cm^{-1} correspond to carbonyl stretching vibrations of carbonyl and carboxylic groups. Bending vibration of –CH2 group of the PUI has been confirmed by band absorbed around 1475 -1460 cm^{-1}. Asymmetric stretch vibration of N-CO-O is observed from the band 1250-1240 cm^{-1}. The band in the range of 1100 -1050 cm^{-1} corroborates the stretching vibrations of N-CO-O and C-O-C moiety of the resultant PUI products.

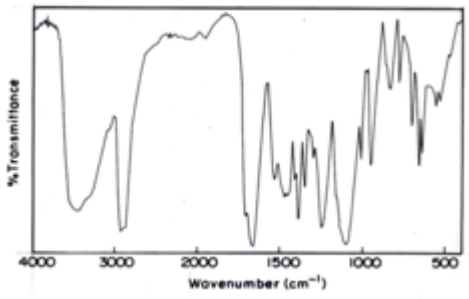

Fig. 1 FTIR of PUI based on TDI.

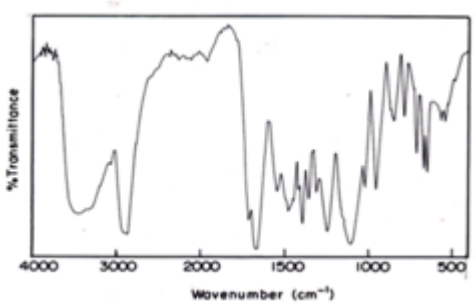

Fig. 2 FTIR of PUI based on HMDI.

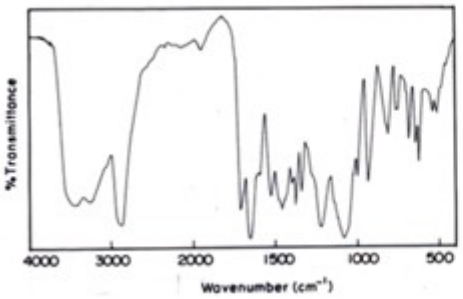

Fig. 3 FTIR of PUI based on H12MDI.

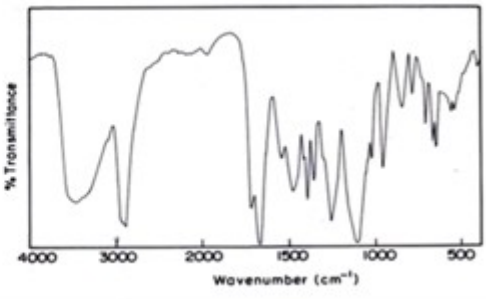

Fig. 4 FTIR of PUI based on IPDI.

4.2. FT-NMR Data

FT-NMR data implies the characteristic feature of the ionomer products [22,23]. NMR spectra of polyurethane samples were recorded using DMSO-d6 as a solvent for the PUI samples formulated from the list of diisocyanate reagents analysed to study the composition of materials. Spectral analyses of ^{1}H NMR and ^{13}C NMR data of these PUI samples have shown the characteristic peak values attributed to the urethane linkage formed for each specific sample. The peak integration observed from the NMR data corroborates the existence of each precursor involved in the reaction of PUI formation.

4.3. Particle Size Analysis of PUI

PUI developed are mostly used as coatings in medical devices. To determine [11,24] the type of coating requirements, particle size and viscosity are important parameters. Smaller particle size is necessary to penetrate into the substrate. For surface coatings, large particle size is preferred to ensure faster drying and suitable viscosity range is essential to avoid sagging (in case of low viscosity) and practical difficulty in application (encountered with high viscosity). In case of PUI, ionic content is inversely proportional to molecular weight and directly proportional to the particle size. As the molecular weight increases, there is reduction in ionic content. It is remarkable to observe the dual effect of the ionic content on PUI particle size. Since the ionic content plays a major role, it is preferred to have PUI of reduced particle size. PUI prepared from IPDI exhibits larger dimension particles compared to the particles from TDI (0.1-10μ). This shows good agreement with GPC data observed from Table 2 in which low molecular weight for the PUI from TDI while the high molecular weight is noticed with case of IPDI shown in fig. 5.

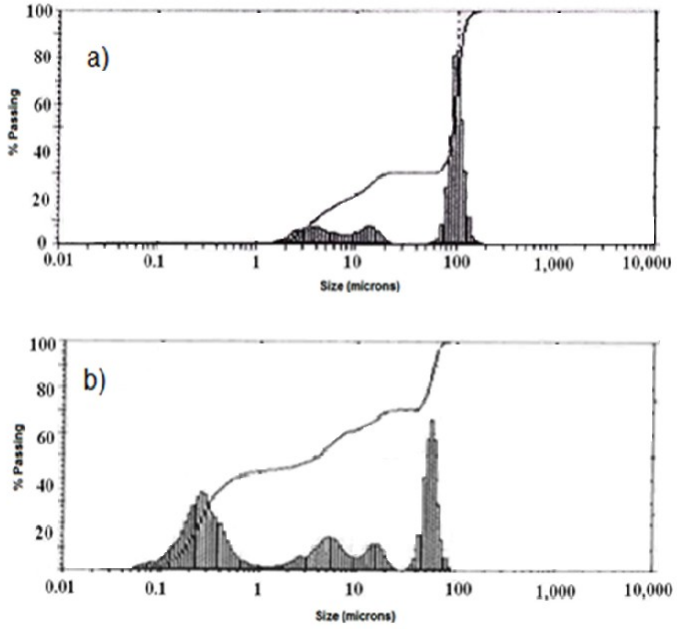

Fig. 5 Particle size analysis of PUI from (a)TDI and (b) IPDI.

4.4. Gel **Permeation Chromatography (GPC)**

The molecular weights (Mn and Mw) of these polymeric ionomers were determined by gel permeation chromatography. The GPC data obtained for the five different samples of PUI are provided in Table 2. In general, PUI products of high molecular weight employed as good bioadhesives. Although the PUI shown in

entry 4 and 5 are identified as high molecular weight samples, the values of remaining PUI samples fall in optimum range. All these PUI samples could potentially exhibit bioadhesive property. Mw values of all these products were obtained with wide variation [23] from each other according to the rate of reactivity of diisocyante reagents (TDI>MDI>HDI>IPDI>H12MDI). The polydispersity (Đ) values observed in Table 2 increases from 1.46 to 4.11 for the samples mentioned in the decreasing rate of diisocyanate reactivity.

Table 2 GPC Data for the list of PUI Samples.

S. No	Isocyanates	M_n	M_w	$Đ_{SEC}$
1	TDI	25848	37920	1.46
2	MDI	27885	60404	2.17
3	HMDI	28822	74403	2.58
4	IPDI	27450	92904	3.38
5	H_{12}MDI	29678	122082	4.11

M_n = Number average molecular weight; M_w = weight average molecular weight; $Đ_{SEC} = M_w/M_n$

4.5. Haemocombatibility

4.5.1. Thrombogenicity of Polyurethanes

Haemostasis, the spontaneous arrest of bleeding from ruptured blood vessels in broad physiological process. The thrombogenic property of three samples of polyurethane in terms of OD (optical density) values, are presented in Table 3.

Table 3 OD Value at 540 nm for different period of contact.

S. No	Contact time (min)	TDI	HMDI	IPDI
1.	10	0.451	0.332	0.606
2.	20	0.415	0.252	0.46
3.	30	0.345	0.112	0.252
4.	40	0.293	0.094	0.191
5.	50	0.275	0.055	0.111

Fig. 6 shows the enhancement in thrombus formation as the contact time increases from 0 min to 50 min. Time versus optical density graph reveals the variation of thrombogenic activity [25] for each specific sample of PUI materials formulated using TDI, HMDI and IPDI. Among the three PUI samples studied, the desired haemostatic nature of the resulting polyurethane materials could be applied in diffuse surfaces with capillary bleeding, since all the three samples exhibit substantial thrombus formation. As there was a drop in the value of OD over the period of 10 min to 50 min upon contact with blood, the experimental samples are considered as haemostatic agents that enhance coagulation and consequently assist the cicatrisation process of the wound, as reported recently [26].

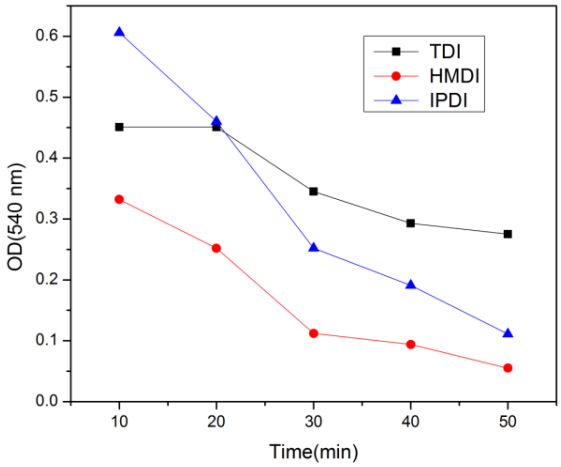

Fig. 6 Thrombogenecity of PUI samples of TDI, HMDI, and IPDI.

4.5.2. Haemolysis Index

The haemolysis index refers to the degree of red blood cells broken during the contact of human blood with PUI sample [27]. High value of haemolytic index indicates more number of red blood cells broken and the low value of haemolytic index revealing the better compatibility of blood with PUI samples [28]. Essentially, the recommended haemolytic index value of PUI material should be less than 5%. According to the ASTM F

756-00, materials can be classified into three categories, depending upon the extent to which they cause the haemolysis: materials exhibiting the haemolytic index value of 0 – 2%, are non haemolytic; 2 – 5% are slightly haemolytic and that with > 5% are haemolytic. Since this value was found to be < 2 % for the untreated form of polyurethanes of this study, it is considered as non-haemolytic. The sample in the untreated form seems to be more recommendable than the treated form. Haemolytic values of PUI samples of three different categories are presented in Table 4. According to the Fig. 7 haemolytic index value less than 2% reveals that the polyurethanes synthesized are non-haemolytic in the untreated form. It is slightly haemolytic for the treated samples and the PBS extraction solution exhibit highly haemolytic.

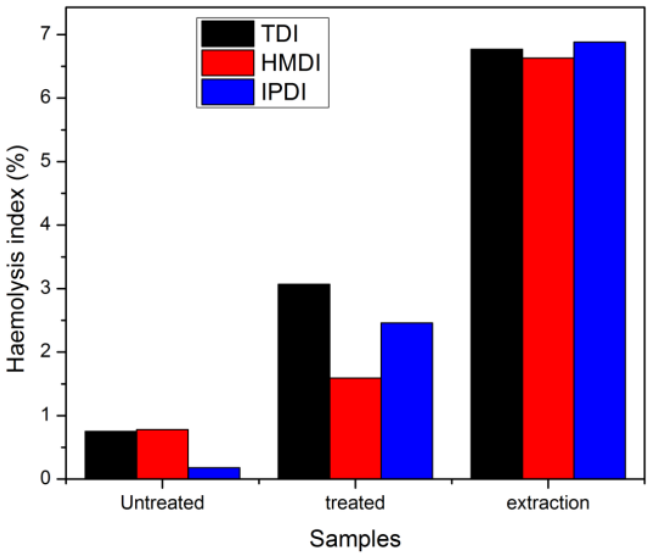

Fig. 7 Hemolysis index of PUI.

Table 4 Optical density values of haemolysis at 540 nm.

S. No.	Sample	OD	Haemolytic index (%)
1.	X	0.605	3.07
2.	Y	0.519	1.59
3.	Z	0.569	2.46
4.	X_1	0.037	6.77
5.	Y_1	0.045	6.63
6.	Z_1	0.031	6.88
7.	X_2	0.384	0.75
8.	Y_2	0.382	0.78
9.	Z_2	0.438	0.18

4.5.3. Platelet Adhesion Test

The platelet adhesion results of all the three PUI specimens obtained from diisocyanates, TDI and HMDI are shown in Fig. 8. The platelet activation and adhesion [29] depend on the surface characteristics and protein adsorption property of a material. Generally, when the blood is allowed to flow through the artificial surfaces, the plasma proteins such as Alb, IgG and FN are adsorbed on the surface, which depends on the characteristics of the polymers themselves. The synthetic polymers are less attractive to proteins than cellulosic materials. Platelets are extremely sensitive cells that may respond to minimal stimulation. Activation causes platelets to become sticky and changes shape into irregular spheres with spiny pseudopods, accompanied by internal contraction and extrusion of the storage granule contents into extracellular environment. These secreted platelet products stimulate other platelets, causes irreversible platelet aggregation and lead to the formation of fused platelet plugs.

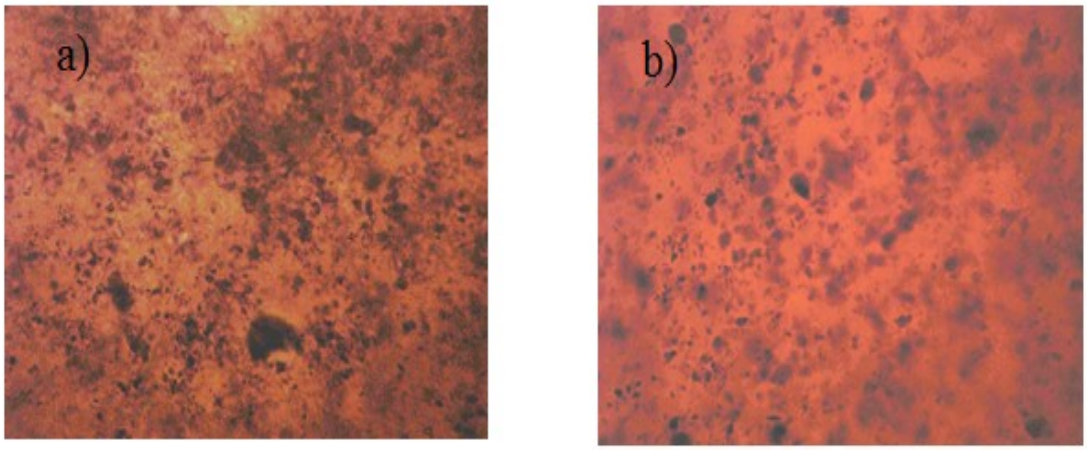

Fig. 8 Optical images of platelets adhesion of PUI from (a) TDI and (b) HMDI (100X).

Subsequently, the platelets release some materials such as Adenosine Diphosphate (ADP), Adenosine Triphosphate (ATP), serotonin and platelet factor 4 (PF4), beta-Thromboglobulin (bTG), FN, vWF and fibronectin and activated arachidonic acid to produce thromboxane A2 (TXA2). Then ADP and TXA2 induce more platelet aggregation on the surface and result in more plugs. Followed by Hagemen factor (factor XII), which is activated to induce the intrinsic pathway, meanwhile the white blood cells release thromboplastin to induce the extrinsic and common pathway. As a result the system leads to the formation of thrombin, a non-soluble fibrin network or thrombus.

Lee et al. reported platelet adhesion in the presence of plasma proteins decreases gradually with increase of surface wettability [30]. However, plasma protein adsorption on a wet surface increased with increase in surface wettability. More plasma protein adsorption on the hydrophilic surface caused less platelet adhesion, probably due to platelet adhesion inhibition proteins such as high molecular weight kininogen, which preferably adsorbs on surface by the so-called Vroman effect. Even though the presence of both the plasma proteins and wet surface influence the platelet adhesion and activation, the porous and surface roughness are also important factors. TDI and HMDI based PUI shown better adherence for platelet, among TDI, HMDI and IPDI, used for analysis.

V. Conclusions

Segmented polyurethane ionomers (PUI) were synthesized from the linear polyol, poly (ethylene glycol), diisocyanates (TDI, MDI, HDI, IPDI and H12MDI) and chain extender, DHBA. PUI films were obtained by two steps have been characterized by FTIR, FT-NMR, particle size analysis and GPC data. These PUI films were proved to be good candidates as bioadhesives on the basis of results inferred from thrombogenicity, haemolysis and platelet adhesion test. In particular, lower value of haemolytic index detected from in vitro study of PUI samples with human blood as well as platelets test results concluded that these PUI materials are biocompatible for application on the wound surface to arrest bleeding without causing any adverse effect.

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