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Hemocompatibility and Surface Morphology of Vegetable oil Based Polyurethane Eggshell Powder Nanocomposite

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ABSTRACT

In the present work, biocompatible polyurethane (PU) and PU–based nanocomposites (PUENs) of different composition have been synthesized from hydroxylated olive oil- based polyurethane and eggshell powder as filler. The synthesized polyurethane nanocomposites are characterized by FT-IR spectroscopy, SEM and XRD analysis. The chemical resistance and biodegradability of the PUENs have been studied in different chemical environments. SEM analysis confirms the incorporation of eggshell powder into the polyurethane matrix. The XRD study reveals that the intensity of the peak increases from 90 to 137 cts with the increase in crystallinity. The chemical resistance shows the swelling and degradation of the PUENs at higher concentration of the HCl and NaOH. In soil burial degradation method 13%-18% of weight loss is observed in PUEN3 and PUEN4. The hemolytic rate of PUEN4 is < 2% at 10 to 40µg/mL showing non-hemolysis, implies better hemocompatibility of the sample.

Keywords: Biocompatible, Eggshell, Nanocomposites, Olive oil, Polyurethane.

INTRODUCTION

Nowadays, Nanocomposites are suitable materials to meet the emerging demands arising from scientific and technologic advances[1].There is an expanding impact on the substitution of synthetic reinforcements in composites by natural fiber/ particles. It is due to their renewability and low environmental impact[2]. The properties of composites depend on filler size/shape, filler content and interfacial adhesion. It will cause the behaviour of filled polymers to be more complex than their unfilled counterpart[3]. Vegetable oils such as castor oil, olive oil, soybean oil, sunflower oil, etc. have been used as a raw material for the synthesis of biopolymers. The fatty acid chains of the vegetable oils are modified by introducing the functional group such as hydroxyl, epoxy or carboxyl groups for the production of biopolymers[4]. Vegetable oils like castor oil are important in our daily life for the production of polymers[5].

Vegetable oil based PU plays an important role such as flexible, rigid, elastomers, surface coatings and adhesives[6]. A bio composite is formed by the polymer matrix (resin) and reinforced with natural fibers usually derived from plants, cellulose or particles like $CaCO_3$, talc, etc.[7]. Animal fibre or eggshells are considered to have potential use as reinforcing agents in which provides good strength and stiffness[8]. Eggshell contains 94% of calcium carbonate, 1% of magnesium carbonate, 1% of calcium phosphate and 4% of organic matter are the chemical compositions[9].

Polymer based eggshell composites are used as a biocompatible polymer for the development of a structurally stable casing for prosthetic devices[10]. The eggshell powder composite have been used to improve the properties when compared with adding commercial calcium carbonate[11]. Recent studies have shown the presence of the natural fillers in biodegradable polymers may strongly accelerate the biodegradation process. The presence of natural fillers increases the water absorption, which highly influences the biodegradation process of the composites, in comparison to neat polymer[12]. PU is widely applied to the field of medical devices and implants such as catheters, heart valves, cardiovascular devices, artificial organs and commercial blood contact materials[13]. Blood compatibility is applied in implantable biomaterials such as artificial blood vessels and orthopedic implants. The development of blood contacting materials is necessary to improve the hemocompatibility by surface modification[14]. When blood contact with a foreign biomaterial surface, adsorption of plasma protein takes place. The adsorption of albumin has been inhibiting coagulation cascade. The hemocompatibility is the adsorption property of albumin for plasma proteins[15]. The hemocompatibility is influenced by the electronic structure of biomaterial which prevents the transfer of electrons from fibrinogen to the surface[16].

In this study biocompatible polyurethane film is synthesized using a polyol derived from olive oil and Diphenyl methane 4,4' diisocyanate (MDI) and it is used as the matrix for eggshell polymer nanocomposites for the first time. The composites have been characterized by their physical and biocompatible properties.

MATERIALS AND METHODS

Materials

The monomer olive oil was purchased from MarbilYag San.Tic A.S,IZMIR TURKEY. Hydrogen peroxide and Formic acid were received from High Purity Laboratory Chemicals, Mumbai. Diphenyl methane 4,4'- diisocyanate (DPMI),dichloromethane (Solvent), sodium hydroxide, and hydrochloric acid were obtained from M/S Merck (Germany). The initiator Benzoyl peroxide was received from Spectrochem (Mumbai). The cross-linking agent Ethylene glycol dimethyl acrylate (EGMA) and the catalyst Dibutyltin dilaurate (DBL) were purchased from Sigma Aldrich. Distilled water was used for the synthesis of polymer and the preparation of acidic and basic solutions.

Preparation of Eggshell Powder (ES)

Eggshells were washed thoroughly with tap water followed by distilled water. The adhering membrane was removed manually and the shells were dried at room temperature (30°C). The dried shells were powdered and stored in an air tight container at room temperature (30°C) for further use.

Preparation of Hydroxylated olive oil

About 30 mL of olive oil was taken in a 100 mL beaker with 25 mL of hydrogen peroxide. The solution was heated with constant stirring for 1h. Then 25 mL of formic acid was added drop wise with constant stirring for 5h at 60°C.

Preparation of Polyurethane eggshell powder nanocomposites (PUENs)

Polyurethanes were synthesized using Diphenyl methane diisocyanate (NCO) and hydroxylated olive oil (OH) in the ratio of (NCO: OH) 1:2 (w/w). It was taken in a three necked 250 mL flask and stirred at room temperature to get a homogeneous solution using dichloromethane as solvent. Benzoyl peroxide was used as an initiator and Dibutyl tin dilaurate as a catalyst. Ethylene glycol dimethyl acrylate was used as crosslinking agent. The reactants were carried out at 40°C with constant stirring for two hours. The solution was poured into a glass mould of 12 cm × 12 cm and allowed to dry at room temperature. Then it was cured in an oven at 80°C for 24 hours. PUENs were prepared by adding eggshell powder at different concentrations 1 to 4% (w/w) given in Table 1.

Table 1: Synthesis of polyurethane eggshell nanocomposites

Sample code	Ratio of (NCO:OH)	Percentage of eggshell powder (w/w)
PU	1:2	-
PUEN1	1:2	1
PUEN2	1:2	2
PUEN3	1:2	3
PUEN4	1:2	4

Hemolytic assay

Hemolytic assay was studied using human

blood, which was treated with 3.8% sodium citrate and washed well with 0.9% sodium chloride saline solution. The cells were centrifugated at 1500 rpm for 5 minute. The erythrocytes were washed with phosphate buffer saline (PBS), pH 7.4 for 5 minutes. Calculated amount of erythrocytes suspensions (100µL) was added with various concentrations of PUEN4 and incubated at 37°C water bath for 60 minutes. Then it was centrifuged at 1500 rpm for 5 min and the supernatant was collected. The concentration of the hemoglobin was measured in triplicate at 540nm using spectrophotometer and the standard deviation was calculated.

Hemolysis (%) Formula: Test OD/Control OD X 100–Negative control Characterisation

FTIR Spectroscopy

The Fourier Transform Infra-Red spectrometer was used to find the structure of polyurethane matrix and to verify the final chemical structure of the polymer nanocomposites. Infrared spectra were taken in the Fourier Transform Infrared Spectrometer (FTIRaffinity 1 Schimadzu). The spectra of the samples were recorded in the range 4000 cm^{-1} 400 cm^{-1}.

Crystallization behaviour

The crystallization behaviour of the polyurethane nanocomposite films were measured by X-Ray Diffraction analysis (XRD). It was performed a X'PertPro-Panalytic diffractometer with monochromatic Cu-kα radiation at wavelength (λ) of 0.15406 nm, in a range of 20 to 80° (2θ). The crystalline size was calculated using Scherrer's equation.

$$D=K.\lambda/\beta\cos\theta$$

Where, K-shape factor, β-full width at half maximum (FWHM).

Surface morphologies

Surface morphology of polyurethane nanocomposite films was observed in Quanta FEG–250 SEM which permits to achieve a resolution of 1.4 nm even at 1kv electron landing voltage.

Chemical resistance test

Chemical resistance test for the PUENs was carried out according to ASTM C267. A thin film of PUENs is tested in different chemicals at regular time intervals for 72 hours. The percentage of weight loss of PUENs in Sodium hydroxide and Hydrochloric acid were studied.

Soil burial degradation test

Soil burial degradation test was done by ASTM G160 method. PUENs films 15 mm x15 mm x1 mm were buried in common soil at 7 cm depth for 6 months. The normal temperature was 30± 5°C and the humidity was 50-60°C. The samples were removed from the soil once in 7 days to access the changes in their weight loss and surface damage if any.

RESULT AND DISCUSSION

FTIR Spectroscopy of polyurethane eggshell composites

The FTIR spectrum of hydroxylated oil Fig.1 (a) shows a strong peak at 3479 cm^{-1}, indicates the presence of OH group in the molecule. The sharp peak at 1735 cm^{-1} is due to the presence of carbonyl bond C=O. In Fig. 1. (b) the disappearance of OH group confirms the formation of polyurethane. The characteristic absorption peak at 3356 cm^{-1} is attributed to the NH stretching vibration of polyurethane. The presence of the amide group is characterized by absorption at 1527 cm^{-1}. In Fig. 1(c) the stretching vibration at 1411 cm^{-1} and 721 cm^{-1} is corresponding to the carbonate group of eggshell powder. The peak at 1598 cm^{-1} has been assigned to the aromatic ring in the polymer structure. The bands at 2924 cm^{-1} and 2852 cm^{-1} are assigned to the CH stretching vibrations.

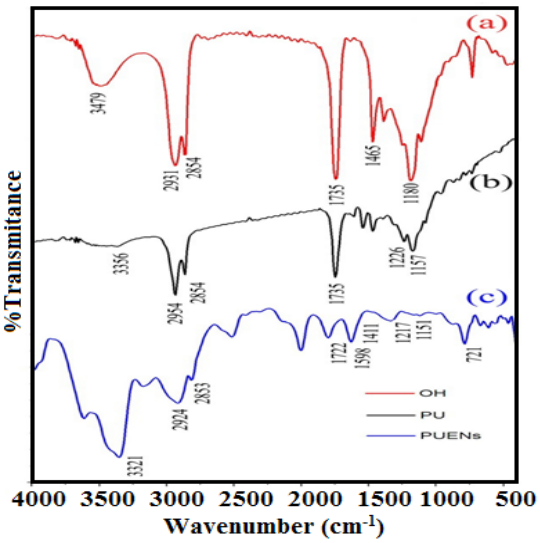

Fig. 1. FTIR spectrum of a) Hydroxylated Olive oil (OH) b) PU c) PUENs

Table 2: FTIR assignment of Polyurethane eggshell nanocomposites

Assignment	Wave number (cm^{-1})		
	HO	PU	PUENs
OH group	3479	-	-
NH group	-	3356	3321
CH2 group	2931	2954	2924
C-H group	2854	2854	2852
C=O in esters	1735	1735	1722
Terminal CH_3 group	1465	1226	1217
Carboxyl group of acids	1180	1157	1151
Carbonate	-	-	1,411,721

X-Ray Diffraction Analysis

XRD analysis is performed to access the status of eggshell nanoparticles and crystallinity within the polymer matrix. The XRD spectrum of eggshell powder (Fig. 2. (a) has a well-defined sharp peak at 2θ= 29.5 depicts the 100% crystallinity of the ES nanoparticles. The minor peak at 23.2, 31.5, 36.1, 39.5, 43.2, 47.2, 47.5 and 48.5 is due to the presence of calcium carbonate. The particle size of eggshell powder is found to be 55nm. The XRD pattern of the synthesised olive oil - based polyurethane (PU) is given in Fig. 2. (b) Shows a peak at 2θ=19.74, has 72% of crystallinity. The crystallinity of the PUENs samples increases from 73.2 to 74.6% by the addition of eggshell nanoparticles. The eggshell powder is highly intercalated with the polymer matrix. The percentage of crystallinity increases with the increase in percentage of eggshell nanoparticles. The height of the peak increases from 90 to 137 cts and the angle of reflection shifted to lower angles. The diffraction angle for PU is observed at 2θ=19.74 when compared to 18.87 A0 for PUENs suggesting the formation of PUENs.

Table 3: XRD analysis of the PUENs

Samples	Percentage of crystallinity	Crystallinityindex
PU	72	0.61
PUEN1	73.2	0.63
PUEN2	73.3	0.63
PUEN3	74.3	0.65
PUEN4	74.6	0.66

Scanning Electron Microscope Analysis

In the SEM images gray colored regions shows the polymer matrix, and the brighter spots represent distribution of eggshell nanoparticles. In Fig. 3 (a) eggshell nanoparticles shows a smooth surface with spherical dots. In Fig. 3(b) shows an irregular fold of the extended chain structure of hard and soft segment polyurethane. It is noted that at lower percentage facilitates the formation of clustered and aggregated form of ES nanoparticles (PUEN1 and PUEN2). The higher ES content the nanosized particles are almost uniformly dispersed in the polymer matrix (PUEN3 and PUEN4).

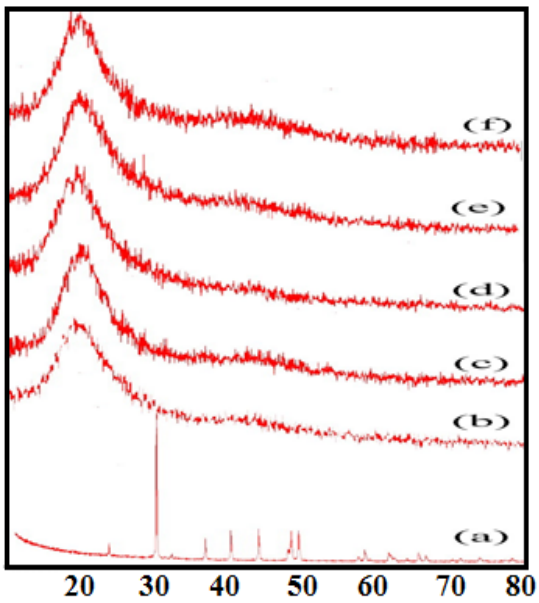

Fig. 2. XRD patterns of a) egg shell powder b) PU c) PUEN1 d) PUEN2 e) PUEN3 and f) PUEN4

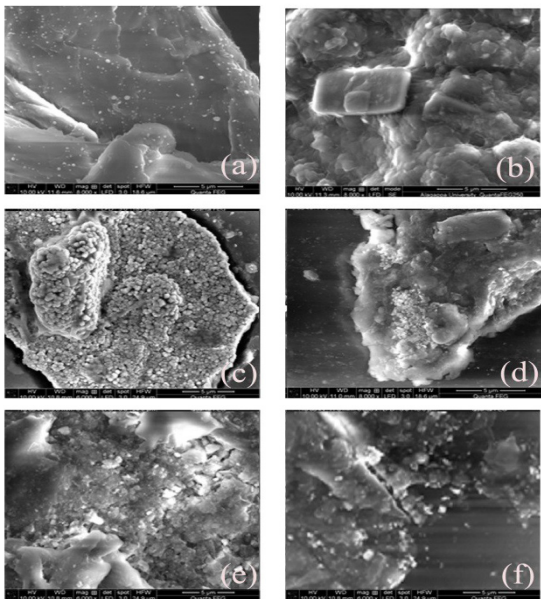

Fig. 3. Surface morphology of a) eggshell powder b) PU c) PUEN1 d) PUEN2 e) PUEN3 f) PUEN4

Chemical resistance test

The chemical resistance test for polyurethane eggshell composites have been

analyzed in 5%, 10%, 15% of sodium hydroxide and hydrochloric acid respectively. Significant percentage of weight loss has been observed in PUEN3 and PUEN4, because of the presence of high percentage of eggshell powder. Thus the percentage of eggshell nanoparticles increases with the increase in weight loss.

Table 4: Percentage of weight loss in chemical resistance test

Samples	NaOH (%)			HCl (%)		
	5	10	15	5	10	15
PU	0	1	1	0	0	1
PUEN1	1	1	1	0	0	1
PUEN2	1	1	1	0	0	1
PUEN3	1	1	2	1	1	2
PUEN4	1	2	2	1	1	2

0–un affected, 1–swelling and 2– degradation

Soil burial test

The percentage of weight loss of the samples PU, PUEN1, PUEN2, PUEN3 and PUEN4 are 2%, 9%, 11%, 13% and 18% respectively. The pH range is 6.9.The percentage of weight loss is found to be higher in PUENs than in plain polyurethane. This is due to the presence of organic matter in the eggshell powder which helps the attack of microorganisms. The presence of eggshell nanoparticles accelerates the degradation of the PUENs. The degradation of the samples increases with the increase in the amount of eggshell nanoparticles. The degradation of PUENs is may be due to the nature of the substrate, degree of polymerization and nano eggshell powder.

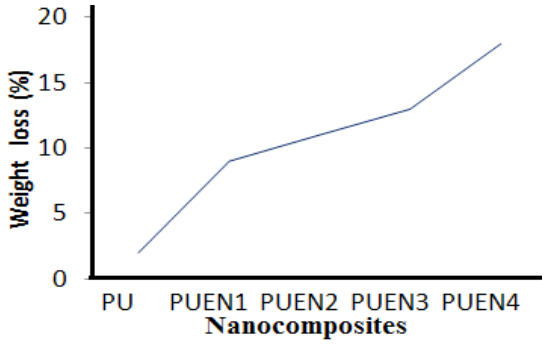

Fig. 4. Soil burial test for PUENs

Hemolysis test

The hemolysis test is used to identify the hemolytic effect of a test sample. The advanced hemolysis test shows that, hemolysis rate is less than 5% indicates the better hemocompatibility. The American chemical society for testing and materials explains that if the hemolysis range is less than 5% denote hemolysis. 2-5% slightly hemolysis and < 2% non-hemolysis. The percentage of hemolysis is < 2% at 10µg/mL to 40µg/mL showing non-hemolysis, implies better hemocompatibility of the sample. Slight hemolysis upto 4.02% occurs at higher concentration.

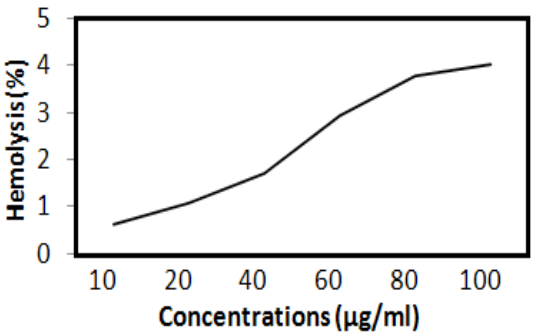

Fig. 5. Hemolysis activity of PUEN4

Table 5: Hemolysis activity of PUENs

S. No	Concentration of the PUEN4 sample (µg/mL)	Hemolysis (%)
1	100	4.02 ± 0.55
2	80	3.78 ± 0.22
3	60	2.94 ± 0.21
4	40	1.71 ± 0.33
5	20	1.08 ± 0.24
6	10	0.63 ± 0.07

CONCLUSION

Polyurethane eggshell nanocomposites have been prepared by adding hydroxylated olive oil with diphenylmethane diisocyanate and nano-sized eggshell powder. The FTIR spectra proved the functionality of the isocyanate is completely converted into urethane. The presence of eggshell particles is also detected in the FTIR spectrum of the polyurethane nanocomposites. XRD results successfully shown that eggshell nanocomposites are reinforced within the polyurethane matrix. The crystallinity of polyurethane is increased by adding the eggshell nanoparticles. SEM images showed the dispersing of eggshell nanoparticles in the polyurethane. The chemical resistance of PUENs shows small variation in the weight which causes swelling and degradation in HCl and NaOH. In soil burial test a partial or slight change in weight is observed in PUENs samples. The polyurethane eggshell composites are having permissible hemolysis rate, shows the good hemocompatibility.

Hence the newly developed PUENs are having slow degradation and good biocompatibility is suitable for medical applications.

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Conflicts of Interest

The authors declare no conflict of interest.

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