ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Biodegradation and Cellular Toxicity Studies of the Poly(Ethylene Glycol)-Sebacic Acid Polymers

Mayson H. Ziboon, Afrodet A. Saleh, *Hadi S. Al-Lami
Department of Chemistry, College of Science, University of Basrah-Iraq

Manuscript Info

Manuscript History:

Received: 11 December 2013
Final Accepted: 19 January 2014
Published Online: February 2014

Key words:
Poly(sebacic anhydride), Biodegradation, Cytotoxicity,

Corresponding Author

Hadi S. Al-Lami

Abstract

Poly(sebacic anhydride) was used to prepare poly(ethylene glycol)-sebacic acid polymers with carboxylic end groups and well defined molecular weight of poly(ethylene glycol). They were purified and characterized by infrared spectroscopy.

In vitro biodegradability studies were carried using wt% loss method on samples in the form compact discs at constant body temperature (37°C) in human plasma. The results revealed that biodegradation needed nearly three months to get 90% hydrolysis; this was defiantly attributed to the poly(ethylene glycol) molecular weight differences in the prepared polymers.

Biocompatibility tests were carried out to represent *in vivo* biodegradation using human blood which is called cellular cytotoxicity method. All prepared polymers showed no toxicity compared to the reference control and to the toxicity of sebacoyl chloride

.

Introduction

Recent research articles are concentrated on biopolymer materials due to their compatibility with the body fluids and have no toxicity[1,2]. They used in the foods, packing up, drugs for both internal and external uses in the body; biopolymers were used in more applications in medicals and pharmaceuticals, commercialism and as cosmetic materials[3], for example, poly(ethylene glycol) which consider as an important commercial polymer for its biodegradable ability[4,5], so it is widely used in biomedical applications[6,7], for its perfect physical and chemical properties as nontoxic hydrophilic polymer, able to dissolve in water and in other organic solvents and for its excellent biological properties[8,9,10].

The fast development in the scientific research and technologies, especially in the field of the cell, cell physiology and biochemistry, lead the scientist to discover the effect of some toxic materials on both molecular and cellular levels. This assists to understand the mechanism of cytotoxicity on the cell especially after use the tissue culture to study the effect of these toxins[11].

The cytotoxicity science was used to study the toxic effect of some materials on the cellular matter, and there are several methods used to evaluate the toxicity of some materials, both *in vivo* and *in vitro*, and study its effect on the cell which represent the essential unit in the body, and the highest responsibility for these toxic materials can be shown by changing in the rate of the cell metabolisms, gene transcription and growth.

There are several changing can be done on the cell function due to the effect of these toxic compounds, so it will be easy to dedicate what can be done by follow up the mechanism of the toxicity on the cellular level[11,12].

In this work, some biodegradable ester polymers were prepared; ester groups represent the linking unit of the polymer chains. These type of polymers are preferred for their medical and pharmaceutical application[14،13], they can be hydrolyzed in vivo and in vitro, highly biocompatible, nontoxic, and can be released outside the body without any side effect as well as they can be configured in many easy features.

Experimental Work

Preparation of (Sebacic Anhydride)

Sebacic acid (10.4 g, 1.1 mole) was dissolved in 150 ml dry toluene preheated up to 65ºC in three neck round bottom flask supplied with mechanical stirrer and condenser. Triethylamine (13.08 ml, 2 mole) was added followed by addition of (10 ml, 1 mole) sebacoyl chloride drop wise to reaction mixture with stirring for 6 hours. Then, 50% v/v of dry ethyl ether was added followed by filtration to separate the triethyl ammonium salt. The filtrate was rotary evaporated under vacuum. The product was white solid with 89% yield. and it was characterized by infrared spectroscopy. Scheme (1) illustrates the chemical reaction.

Preparation of Poly(Ethylene Glycol)-Sebacic Acid Polymer

Poly(ethylene glycol) having different molecular weight (400, 10000 and 20000 g/mole) was refluxed with poly(sebacic anhydride) in the melt form at 180ºC. The product was dissolved in dichloromethane then it was obtained by reprecipitation from diethyl ether as non solvent. The white solid polymer in about 76% yield was dried and characterized by infrared spectroscopy and end-group analysis for molecular weight determination. Scheme (2) shows the polymerization reaction.

Preparation of Phosphate buffer solution) PBS)

Phosphate buffer solution was prepared by dissolving 4.710 g of KH_2PO_4 and 19.778 g of Na_2HPO_4 in 1000 ml distilled water, then the pH was adjusted to (7.4) [14, 15].

Measurements of *In Vivo* Biodegradation by % Weight Loss Method

The polymer samples were hydraulically pressed in the form of disc (10 mm in diameter) using about 0.2 g from each polymer. These discs were fully immersed in phosphate buffer solution (pH=7.4) prepared for this purpose at body temperature (37ºC). The %weight loss was measured at different time using the following equation[16,17]:

$$(\%)\text{Weight Loss} = \frac{Wo - Wt}{Wo} * 100$$

Where:

W_o= Initial weight of the disc,

W_t= Weight of biodegradable disc at time (t).

Biocompatibility Test Using Cytotoxicity *In Vivo* Method

Biocompatibility test was carried out for the prepared polymers against human fresh blood according to the following method[18]:

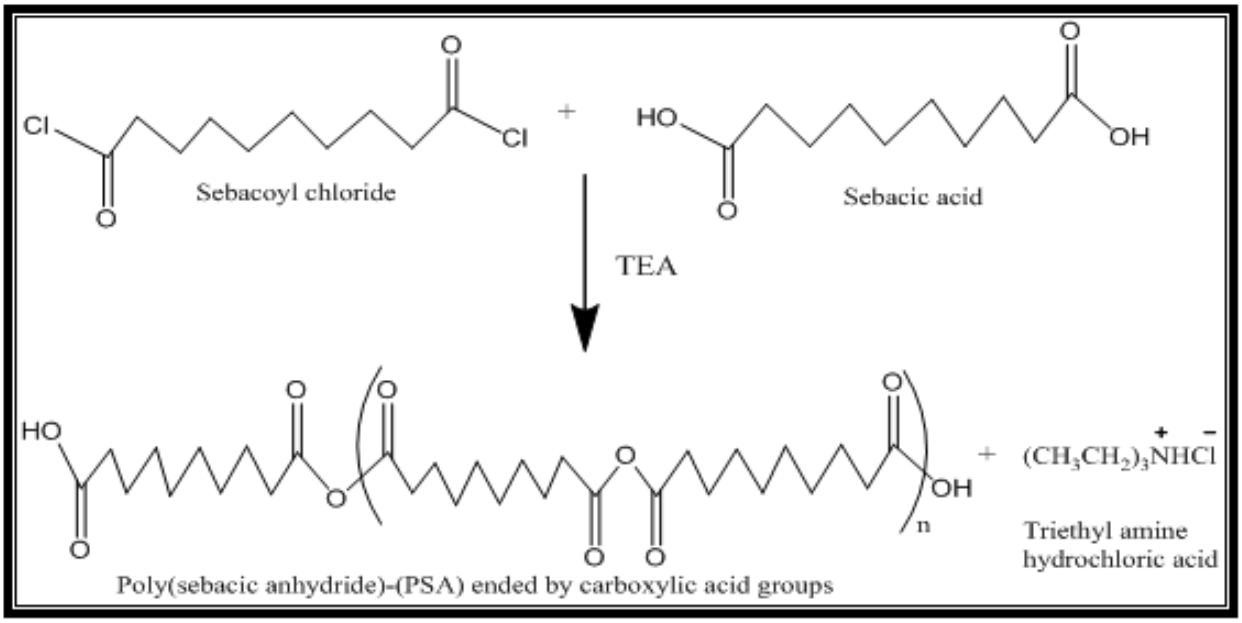
1- Blood solution was prepared by mixing 1 ml of fresh human blood with 20 ml of normal saline.
2- Different concentrations of the polymers were prepared using DMSO as control solution (200, 100, 50, 10, 5 and 0.5 ppm).
3- Each 2 ml of the blood solution was separated on several tubes then 100 µl of each polymer concentration was added to each tube.
4- The tubes were left at room temperature and the formation of the turbidity of the blood solutions was taken as an indication for the cytotoxicity of the prepared polymers. They were tested after 15, 30 and 60 minutes.

Results and Discussion:

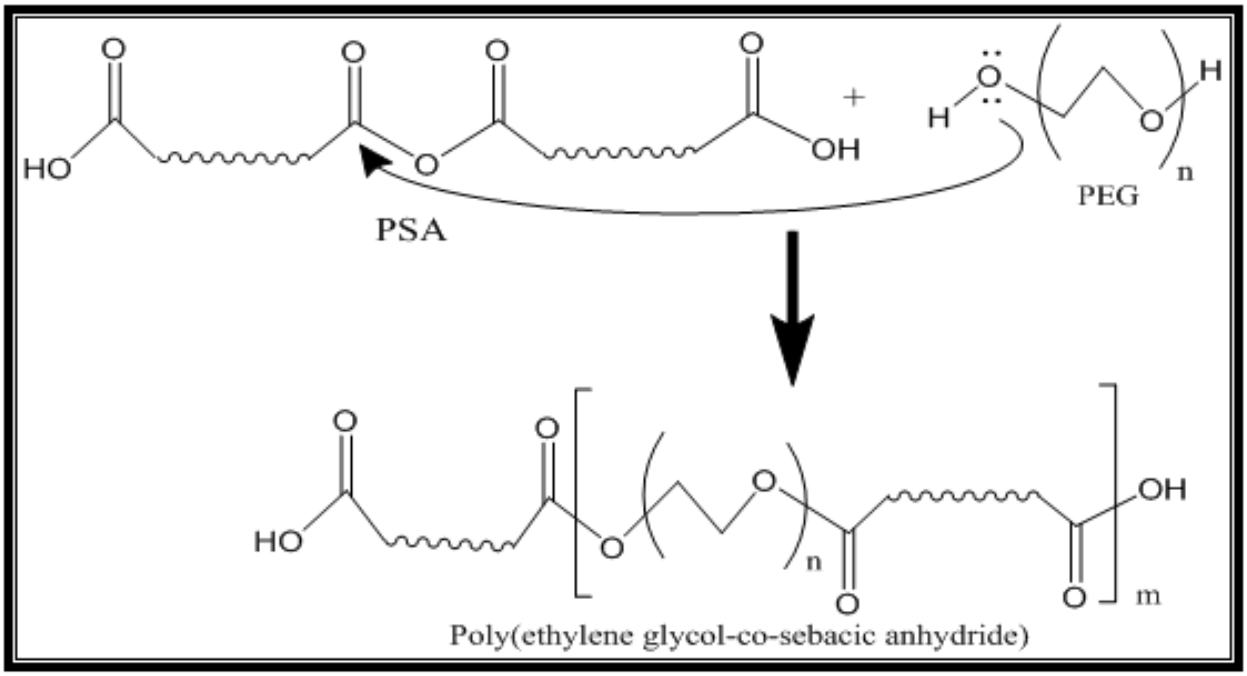
Characterization of Prepared Polymers by Infrared Spectroscopy

All polymers prepared in this work were characterized by Infrared Spectroscopy using (Shimadzu FTIR-8400S/Japan) as KBr discs.

Infrared spectrum of poly(sebacic anhydride) prepared from the reaction of sebacic acid and sebacoyl chloride in the presence of triethylamine in 89% yield exhibits the main characteristic peaks summarized in table (1). This implies real anhydride has been synthesized.



Scheme (1): Chemical reaction for preparing poly(sebacic anhydride)



Scheme (2): Reaction of melt polycondensation polymerization of poly(ethylene glycol) and poly(sebacic anhydride)

Table (1): Main infrared characteristics peaks and their assignment for poly(sebacic anhydride) spectrum.

Stretching Frequency (cm^{-1})	Assigned Bond	Functional Group
1735 + 1812	C=O	-CO-O-CO-
1047	C-O	-CO-O-CO-
1704	C=O	-CO-OH
3450-2600	O-H	-CO-OH
2933 + 2871	C-H	$-CH_2-$

This polymer was used for melt polycondensation reaction with poly(ethylene glycol) as comonomer having different molecular weights, namely 400, 10000 and 20000 g/mole. Their infrared spectra showed the well assigned peaks as listed in table (2). It can be concluded that poly(ethylene glycol) is truly hydrolyzed poly(sebacic anhydride) producing sebacic acid molecules attached to the ends of each poly(ethylene glycol) chains.

Table (2): Main infrared characteristics peaks and their assignment for poly(ethylene glycol)-sebacic anhydride polymers spectrum

Stretching Frequency (cm^{-1})	Assigned Bond	Functional Group
809 (400)[1] 1809 (10000)[2] 1805 (20000) [3]	C=O	-CO-O-CO-
1039 (400) 1036 (10000,20000)	C-O	-CO-O-CO-
1743 (400) 1745 (1000) 1740 (20000)	C=O	-CO-O
1697 (400,10000) 1693 (20000)	C=O	-CO-OH
3447 (400) 3468 (10000) 3530 (20000)	O-H	-CO-OH
1105 (400) 1112 (1000) 1109 (20000)	C-O	-C-O-C-
2926-2854 (400) 2926-2856 (10000) 2920-2799 (20000)	C-H	$-CH_2-$

Molecular weight of poly(ethylene glycol): **1**= 400 g/mole, **2** = 10000 g/mole, **3** = 20000 g/mole

In Vitro Biodegradation of the Prepared Polymers

Biodegradation of the poly(ethylene glycol)-sebacic acid having different chain length of comonomer polyethylene glycol was carried out in phosphate buffer solution having pH=7.4 and 37ºC temperature represent the acidity of stomach and body human temperature respectively using %weight loss method. Figure (1) shows the %weight loss of these polymers in different immersion time. The results imply that different time was needed for the polymer to reach more than 50% weight loss depending on the molecular weight of poly(ethylene glycol) comonomer.

It is quite clear that polymer with PEG having 400 molecular weight was degraded faster than the other two (10000 and 20000 g/mole) and needed 60 days to reach 80% degradation, while the polymer with higher molecular weights of poly(ethylene glycol) needed more than 90 days to reach nearly 90% and being the poly(ethylene glycol) with 20000 molecular weight being the best. It is quite obvious from figures (2) that increasing the time of degradation will increases its percent of degradation.

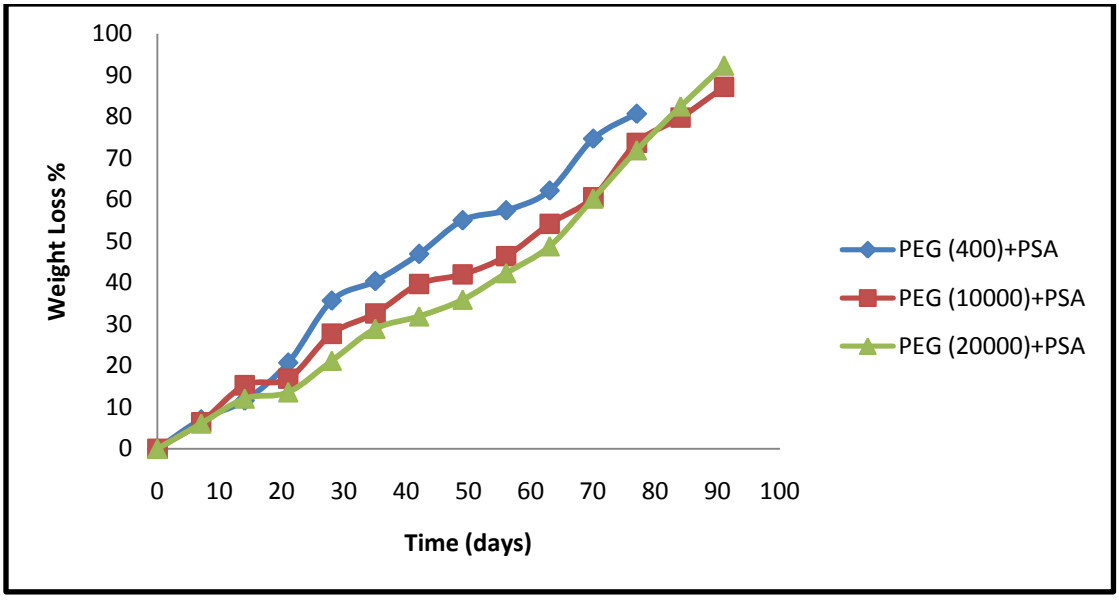
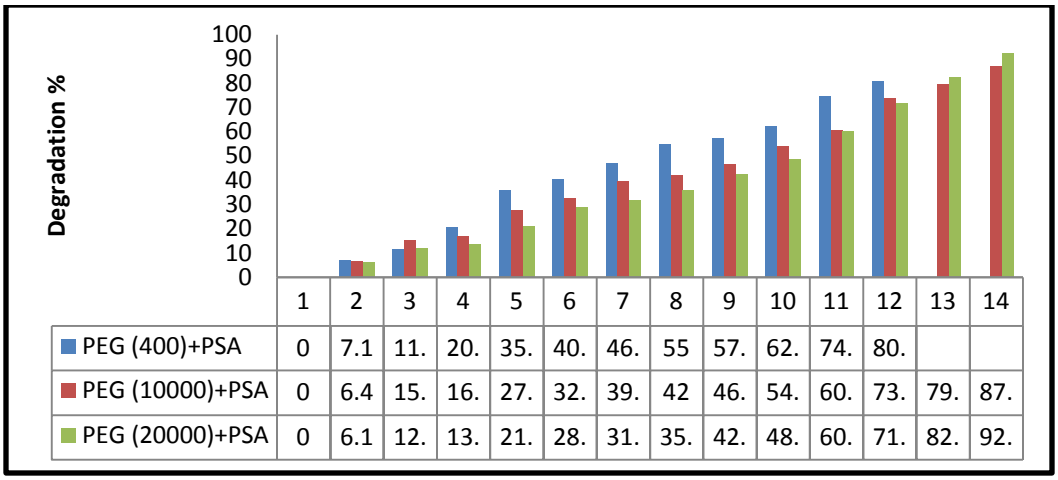


Figure (1): Effect of immersion time on the polymer degradation measured by %weight loss



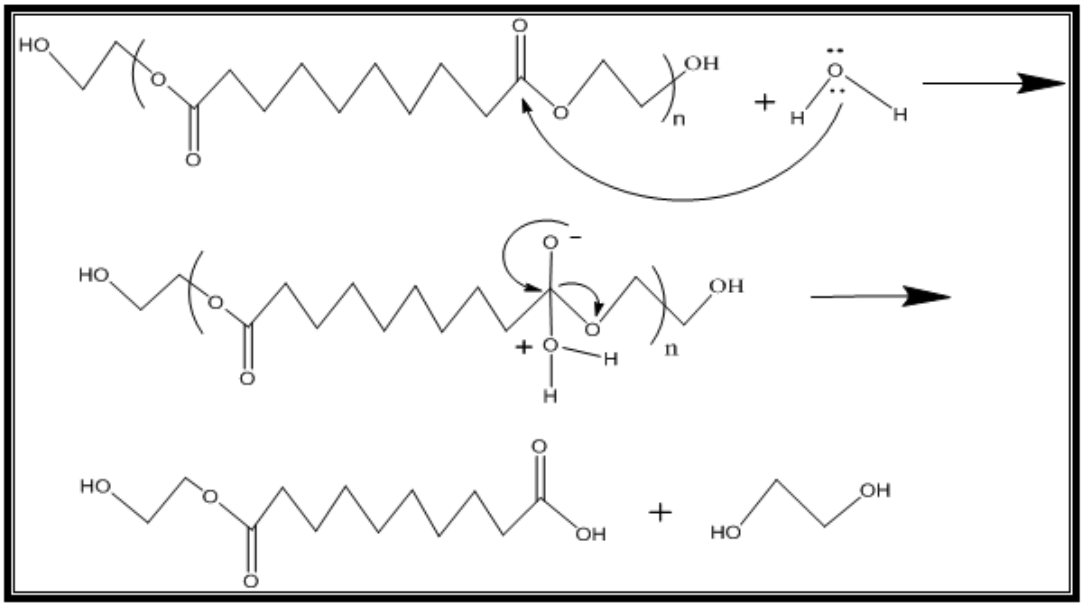
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
PEG (400)+PSA	0	7.1	11.	20.	35.	40.	46.	55	57.	62.	74.	80.		
PEG (10000)+PSA	0	6.4	15.	16.	27.	32.	39.	42	46.	54.	60.	73.	79.	87.
PEG (20000)+PSA	0	6.1	12.	13.	21.	28.	31.	35.	42.	48.	60.	71.	82.	92.

Figure (2): Statistics of polymers degradation

Cellular Cytotoxicity Method

Biocompatibility tests were carried out to represent *in vivo* biodegradation using human blood which is called Cellular Cytotoxicity Method. Table (3) and figure (5) are showing the results of the cytotoxicity of the prepared polymers.

The results revealed that all polymers do not have any toxicity against the human blood solution, that improve their used in both medical and pharmaceutical applications. This is may be attributed to the hydrolysis mechanism of the esteric bond within the main chain of the polymer, in and this is another indicate for previous result of biodegradable, scheme (3).



Scheme (3): Biodegradation mechanisms for the prepared polymers

The biodegradation mechanism as shown above resulted in the formation of dicarboxylic acid monomers, dialcohols and polyalcohol and all these monomers represents the natural metabolic products *in vivo*, for example, sebacic acid is one of the hydrolysis products within the physiological solution of the body, pH=7.4, and its metabolites release acetyl CoA and succinyl CoA were both communicated with Krebs cycle. Acetyl Co A forming isopyrenoid while succinyl CoA consider as the main key for biosynthesis of porfern[19], also the metabolites for polydialcohol and polyalcohol share within the metabolic reaction in the body[20,21,22].

All these results indicate that the prepared polymers can be serve in many medical applications as other homologous polymers[23,24], Polyesters are widely used in medical and pharmaceuticals use such as in surgery fibres, tissue engineering etc.[17,25]. The hydrolysis of polyester represented as mass hydrolysis, while the prepared anhydride polymers can be hydrolysis as type of superficial, so they used as control systems for masterly release of the drugs[19,26].

It can be concluded that the prepared polymers had highly biocompatibility by exhibiting no toxicity and incompatibility against the body fluids. In contrast, sebacoyl chloride monomer showed no biocompatibility with blood solution and that seen from the turbidity appears causing in precipitation of the plasma blood, figure (6).

Table (3): The cytotoxicity of poly(ethylene glycol)-sebacic anhydride polymer

Concentration / Polymer	0.5 ppm	5 ppm	10 ppm	50 ppm	100 ppm	200 ppm
PEG(400)-SA	No toxicity	No toxicity	No toxicity	No toxicity	No toxicity	No toxicity
PEG(10000)-SA	No toxicity	No toxicity	No toxicity	No toxicity	No toxicity	No toxicity
PEG(20000)-SA	No toxicity	No toxicity	No toxicity	No toxicity	No toxicity	No toxicity

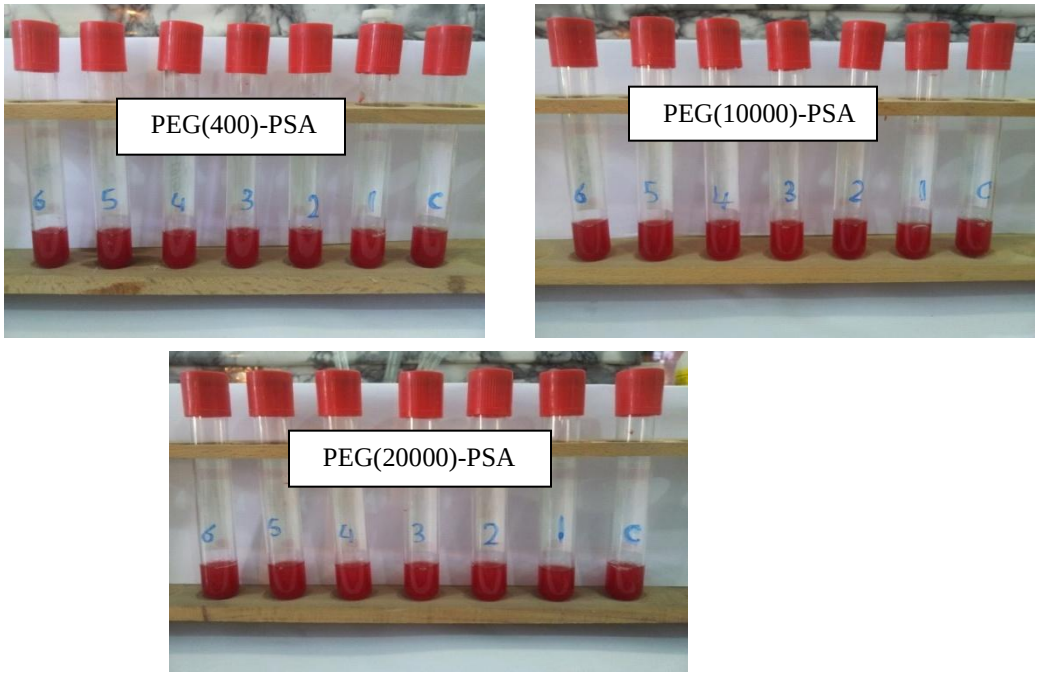


Figure (5): Cytotoxicity of poly(ethylene glycol)-sebacic acid polymers

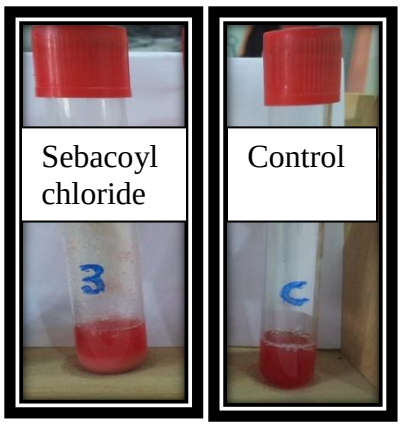


Figure (6): Cytotoxicity of sebacoyl chloride monomer

Conclusions

1. All polymers prepared with good yield.
2. Biodegradations time increasing with increasing poly(ethylene glycol) molecular weight.
3. The ability of the prepared polymer to degraded *in vitro* and *in vivo*.
4. Polymers exhibited excellent biocompatibility with human plasma with no toxicity

References

(1) H.K. Makadia and S.J. Siegel, Poly(lactic-co-glycolic acid as biodegradable controlled drug delivery carrier, Polymers, 3, pp. 377-397, (2011).

(2) J.C. Middleton and A.J. Tipton, Synthetic biodegradable polymers as orthopaedic devices, Biomaterials, 21, pp. 2335-2346 (2000).

(3) D.K .Platt, Biodegradable polymers (market report) , Rapra Technology, pp. 1- 155 (2006).

(4) T. Behjat, A. R. Russly, C. A. Luqman, A. Y. Yus, and I Nor Azowa, Effect of PEG on the biodegradability studies of kenaf cellulose- polyethylene composite, International food research journal, 16, pp. 243-247 (2009).

(**5**) G. Bonacucina, M. Cespi, G. Mencarelli, G Giorgioni, and G.F. Palmieri, Thermosensitive self-assembling block copolymers as drug delivery system, Polymers, 3(2), pp. 779-811 (2011).

(6) V.G. Kadajji and G.V. Betageri, Water soluble polymers for pharmaceutical applications, Polymers, 3(4), pp. 1972–2009 (2011).

(7) S. Kandavilli, V. Nair and R. Panchagnula, Polymers as transdermal drug delivery systems, Pharmaceutical Technology, pp. 62-80, May (2002).

(8) J.L. Ostroha, PEG-Based degradable networks for drug delivery applications, PhD thesis, Drexel University, Philadelphia, USA (2006).

(9) S. Zhou, X. Liao, X. Li, X. Deng and H. Li, Poly(D,L-lactide-co-poly(ethylene glycol) microspheres as potentials vaccine delivery systems, J. Controlled Release, 86: pp.195-205 (2003).

(10) D. Shuwisitkul, Biodegradable implants with different drug release profile, PhD thesis, Freie University Berlin, Germany (2011).

(11) G. Eisenbrand, P.B. Zobel, V. Baker, B. M. Blaauboer, B.J. Boobis, A. Carrere, A. Kevekordes, S. Lhuguenot, J.C. Pieters, and R. Kleiner, Methods of *in vitro* toxicology, Food Chem. Toxicology., 40, pp. 193-236 (2002).

(12) W. Vincour, B. Shosevov, and O. Altman, "Role of plant heat-shock proteins and molecular chaperones in the biotic stress response", Trends Plant Sci., 9, pp. 244-252, (2004).

(13) S.W. Shalaby and K.J. Burg, Absorbable and biodegradable polymers, Bocaraton London, New York Washington D.C. (2005)

(14) L.S .Nair and C.T .Laurencin, Polymers as biomaterials for tissue engineering and controlled drug delivery, Biotechnology, 10 , pp. 47-90 (2006).

(15) M. Kiremitci and G. Deniz, Synthesis, characterization and *in vitro* degradation of poly(DL-Lactide)/Poly(DL-Lactide-co- Glycoside) films, Turk. J. Chem., 23: pp. 153-161 (1999).

(16) A.J. Dong, J.W. Zhang, K. Jiang and L.D. Deng, Characterization and *in vitro* degradation of poly(octadecanoic anhydride), J. Mater. Sci., 19, pp. 39-46 (2008).

(17) J. Yang, A.R. Webb, S.J. Pickerill, G. Hageman and G.A. Ameer, Synthesis and evaluation of poly(diol citrate) biodegradable elastomers, Biomaterials, 27, pp. 1889-1898 (2006).

(18) M.G. Nair, A.R. Mishar, M.H. Muks, W.H. Taff, J.F. Kesller, J.R. Miller, P.P. Zhu, J.D. Meinhart, and D.G. Lynm, Faerifungin a new broad spectrum antibiotic from Streptomycin griseous, Autotrophic US, J. Natural Products, 52, pp. 779-809 (1989).

(19) R. Chandra and R. Rustgi, Biodegradable polymers, Prog. Polym. Sci., 23: pp. 1273–1335 (1998).

(20) G.D. Barrett and M.N. Yousaf, Design and application of biodegradable polyester tissue scaffolds based on endogenous monomers found in human metabolism, Molecules, 14, pp. 4022-4050 (2009).

(21) M .Schmid, A. Ritter, A. Gubelnik M... Zinn, Autoxidation of medium chain length polyhydroxyalkonate, Biomacromolecules. 8, pp. 579-584 (2007).

(22) M. Zinn, H.U. Weilenmann, R. Hany, M. Schmid, T. Egli, Tailored Synthesis of Poly[R]-3-hydroxybutyrate-co-3hydroxyvalerate (PHB/PHV) in Ralstonia eutropha DSM 428, Acta Biotechnologica, 23, 309-316 (2003).

(23) M.L. Hans, Synthesis, characterization, and application of biodegradable polymeric prodrug micelles for long-term drug delivery, PhD thesis, Drexel University, Philadelphia, USA (2005).

(24) K. Patel, J. Porwoll, M. Nakayama, T. Okano and F.M. Winnik, Material matters materials for biomedical applications, Aldrich Materials Science, 3(5), pp.54-87 (2010).

(25) R. Hoogenboom and U. Schubert, Microwave-assisted polymer synthesis: recent developments in a rapidly expanding field of research, Macromol. Rapid Commun., 28: pp. 368-386 (2007).

(26) N. Kumar, M. Krishnan, T. Azzam, A. Magora, M. Ravikumar, D. Flanagan and A.J. Domb, Analysis of fatty acid anhydrides and polyanhydrides, Analytica chimica Acta, 465, pp. 257-272 (2002).