

***In vitro* degradation study of polymers prepared from sebacoyl chloride with (di)ethylene glycol monomers and polymer**

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Abstract

In this study, some ethylene glycol based polymers, namely poly(ethylene sebacate), poly(diethylene sebacate), and poly(ethylene glycol)-sebacate copolymers having three different molecular weights were prepared by condensation polymerization with sebacoyl chloride in very good yields ranging from 81-88%. All polymers designed to be ended with hydroxyl groups. The prepared polymers were characterized by infrared spectroscopy and molecular weight determination using end-groups analysis method and both methods were confirmed the right structures of the prepared polymers.

Biodegradation studies of all the prepared polymers were carried out by pressing the polymer sample in the form of compact disc weighing 0.2 g and about 1 cm in diameter. In vitro biodegradability studies was done using %weight loss of constant body temperature (37°C) in human plasma pH=7.4, and determination of intrinsic viscosity of the solution of the degradable polymers. The results revealed that the poly(diethylene sebacate) polymer needed more than four months to reach only 21% loss while poly(ethylene sebacate) reached nearly 25% in the same period of time. The same behavior was observed in the intrinsic viscosity study.

Under the same conditions, degradation of the polymers produced from condensation polymerization of poly(ethylene glycol) having different molecular weight, i.e. (400, 10000, 20000) g/mole with sebacoyl chloride was also studied by determining of their degradable solution intrinsic viscosities. The results obtained imply that the number of polymeric repeated units has a great influence on the degradation of the polymer, the more repeated unit the less degradation. This is considered as a good sign for these polymers to be used as a drug carrier in controlled drug delivery systems as they increase residence time in the body.

Keywords:

Biodegradability, poly(ethylene sebacate), poly(ethylene glycol), weight loss, viscosity

1-Introduction

Biodegradable polymers are defined as a specific type of polymer that disappeared after its envisioned purpose to result in natural byproduct gases (CO₂, N₂), water, biomass, and inorganic salts, and they are recently widely reviewed [1,2]. These systems are widely used and much of it does not leave residues within the human body, this kind of polymers may be naturally present or artificially made, since these polymers dissolve at different times within a living organism [3,4]. They are mainly prepared by condensation reactions and ring opening polymerization. They may contain different linkage units, such as ester, amide, and ether.

The breakdown of these polymers may occur within or outside of the organism by biological agents such as microbes or enzymes, or by microorganisms such as fungi and bacteria, or change in acid function, and this decomposition process may be inside the organism, called (*in vivo*) or take place outside of the organism, called (*in vitro*), and there are factors that affect the rate of decomposition bio-polymer such as temperature and pH molecular weight function and nature of chemical compositions and others [5]. The surrounding environment of the polymer is important as the polymer structure itself. These factors involved items such as the pH, temperature, microorganisms present, and water as just a few examples [6]. Biodegradation is said to complete when there are no oligomers or monomers left [5].

The process of decomposition occurring is accompanied by a decrease in molecular weight normally, outputs that are either in the form of monomers or simple structures. The biodegradable phenomenon is a dynamic state of interaction between the living tissues and polymer material components which act as a foreign body while planting. Polymer chains ending functional groups may be hydrophilic or hydrophobic. In general, there are three processes that accompany the process of decomposition of polymers, decreasing in molecular weight causing a decrease in polymer strength with time and resulting in losing weight (mass).

The main three areas of application of biodegradable polymers are in medicine, packaging, and agriculture. Their applications were extended to be used not only on pharmacological devices, but as matrices for enzyme immobilization and controlled-release devices, therapeutic devices, as temporary prostheses, and porous structure of tissue engineering [7,8].

Poly(ethylene glycol) (PEG) has been widely used. PEG could be used as absorbent materials in horticulture, healthcare and agricultural applications due to its low solubility and a very important water uptake [9,10], as carriers in drug delivery [11], and temporary matrixes or scaffolds in tissue engineering due to its biodegradability, biocompatibility, good mechanical properties, and molding properties. Poly(ethylene glycol) is often introduced to other monomers for its hydrophilicity, biocompatibility, and non- immunogenicity [12,13]. PEG helps in reducing the aggregation of red blood cells and so improves the blood compatibility of PEG copolymers that are implanted as cardiovascular devices such as stents. It is mainly used in storage of blood and organs and in other surgical repair [2].

In our previous work, poly(sebacic anhydride) was used to prepare poly(ethylene glycol)-sebacic acid polymers with a 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 a 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 [14].

Herein, *in vitro* biodegradability studies for the prepared ethylene glycol based-polymers, namely poly(ethylene sebacate), poly(diethylene sebacate), and poly(ethylene glycol)-sebacate copolymers having three different molecular weights was carried using wt% loss method at a constant body temperature (37°C) in human plasma pH=7.4, and the degradation was followed also by determining of the intrinsic viscosity of the degradable polymer solutions as another method of investigating polymers biodegradability.

2- Materials and Methods

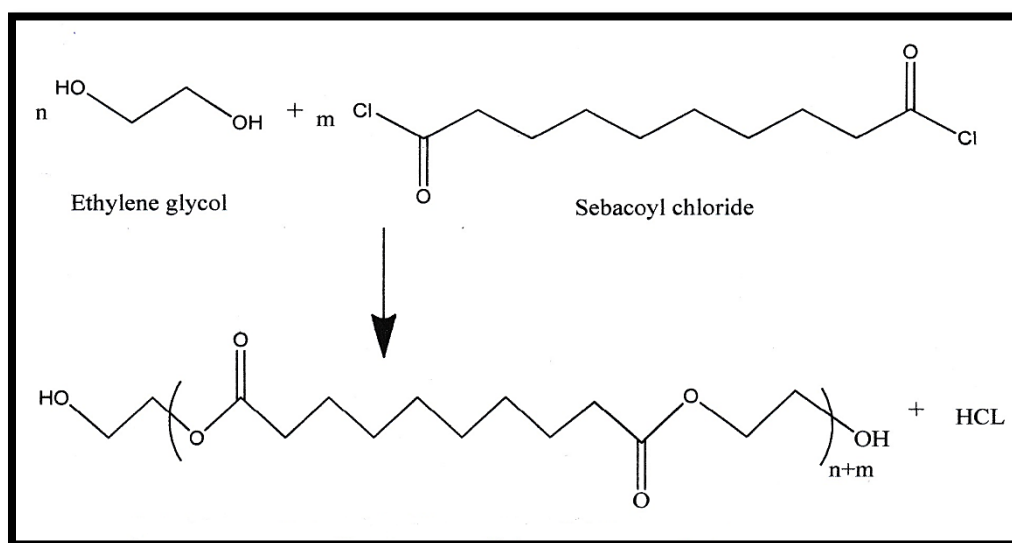
Materials

Toluene, dichloromethane, and diethyl ether were dried by normal procedures [15]. Ethylene glycol, diethylene glycol, sebacoyl chloride, trimethylamine, and three poly(ethylene glycol) polymers having different molecular weights were used as purchased with no further treatment.

Methods

Synthesis of poly(ethylene sebacate), (PES), ended by hydroxylic groups

Ethylene glycol (1.05 mole, 8 g) was dissolved in 100 ml of dry toluene, the volume was reduced to 50ml of toluene by rotary evaporator to remove the adsorbed water molecules on the ethylene glycol, followed by the addition of 50 ml dry dichloromethane and (1 mole, 7.149 ml) of sebacoyl chloride, and then the mixture left stirring for 24 hours [16]. The white solid product washed with dry diethyl ether to remove the formed hydrochloric acid as a byproduct of the condensation polymerization of ethylene glycol and sebacoyl chloride. The product was dried and the yield was about 88%. Scheme (1) shows the chemical synthesis route.

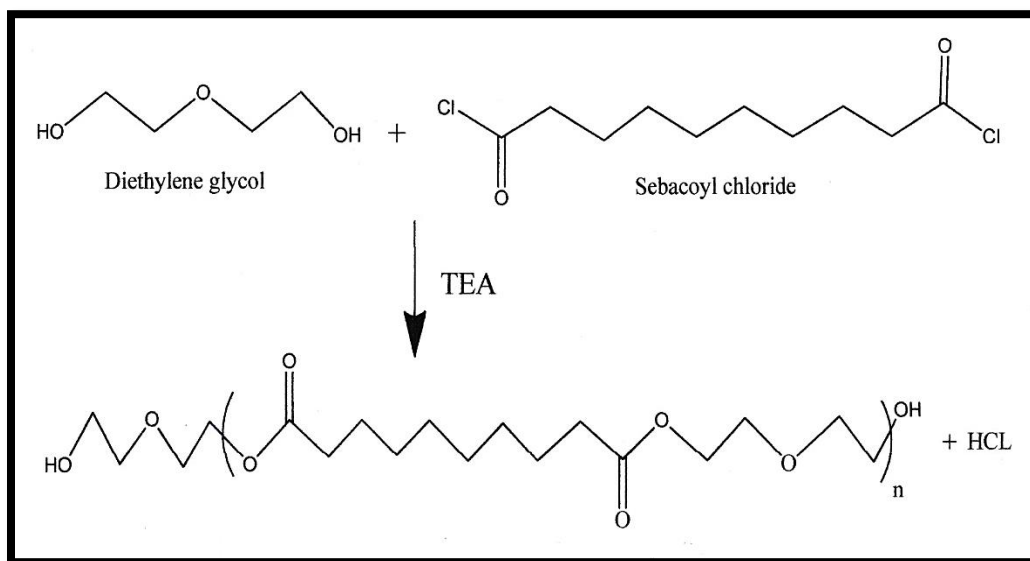


Scheme (1): Synthesis of poly(ethylene sebacate).

Synthesis of poly(diethylene sebacate)

Diethylene glycol monomer (1.05 mole, 8g) was dissolved in dry toluene (100 ml), and the toluene volume was reduced to 50 ml by rotary evaporator to remove the adsorbed water on the diethylene glycol. After that, 50 ml of dry dichloromethane and (2 moles, 9.345 ml) of triethylamine was added, followed by the dropwise addition of monomer sebacoyl chloride (1 mole, 7.149 ml) for a period of 30 minutes in ice bath due to the exothermic reaction, and then the mixture left to stirring magnetically for overnight. At the end of the addition, dry diethyl ether was added and the mixture filtered to remove the byproduct triethylammonium chloride

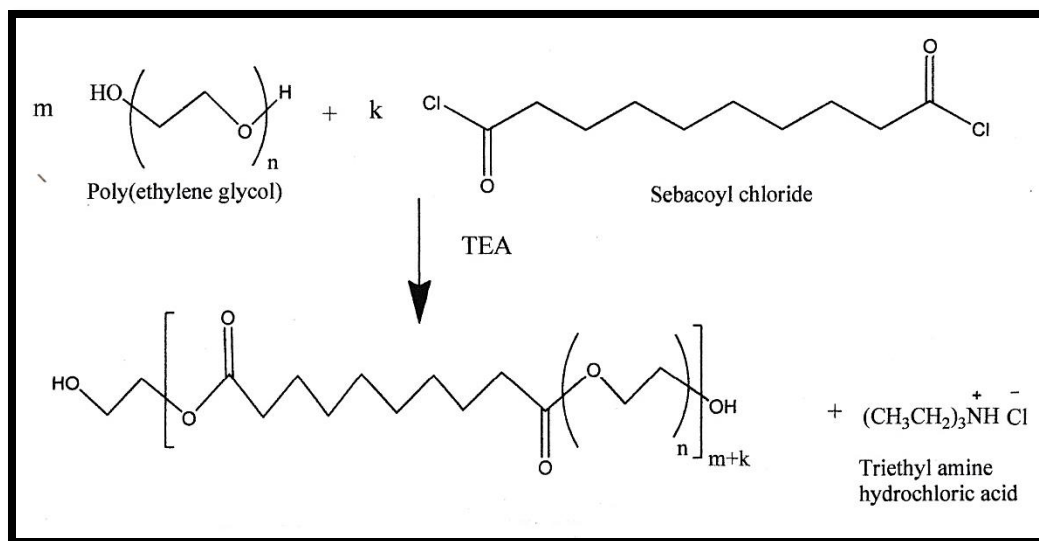
[17]. The solvents were removed by rotary evaporator, and then the whit gummy product washed with dry diethyl ether again to remove the unreacted monomers, the yield was 86%. Scheme (2) represents the polymerization chemical equation.



Scheme (2): Synthesis of poly(ethylene sebacate).

Synthesis of poly(ethylene)-sebacate copolymers

Poly(ethylene glycol), 1.1 moles, dissolved in 300 ml dry toluene, then it was transferred to distillation apparatus to get rid of water molecules adsorbed on polymer by reducing the solvent quantity to the half of its original volume. After that, 2 moles of triethylamine, 3.54 ml, was added to the stirred solution followed by the dropwise addition of sebacoyl chloride (1 mole, 3.73 ml) over a period of 30 minutes, with cooling the mixture with an ice bath [18]. Then, the product washed thoroughly twice with dry diethyl ether to remove triethylammonium chloride salt formed. Scheme (3) the representational scheme of the condensation polymerization, and Table (1) shows the resulting polymers physical state and yield.



Scheme (3): Representative scheme for the synthesis of poly(ethylene glycol)-co-sebacate.

Table (1): Molecular weight of poly(ethylene glycol) used in the preparation of poly(ethylene glycol)-sebacate and their yields

PEG Molecular Weight (g/mole)	Physical State	Yield (%)
400	Viscous	86
10000	Solid	84
20000	Solid	81

Preparation of Buffer Solution, PBS

The buffer solution type phosphate was prepared by dissolving 4.7 g of KH_2PO_4 and 19.78 g of Na_2HPO_4 in one liter distilled water, and the pH was adjusted to 7.4 related to human body temperature [18,19].

In vitro biodegradation measurement by %weight loss

The polymeric samples weighing nearly (0.2 g), for each sample, were pressed in a circular discs form. These discs were immersed in the acidic buffer solution (pH = 7.4) and in the body temperature (37 °C). Then the weight loss by the time of immersion was measured by the following equation [2,20]:

$$\text{Weight Loss (\%)} = (W_o - W_t) / W_o * 100$$

Where W_0 is the initial weight of the polymeric sample disc, W_t is the weight of polymeric sample disc at time t .

In vitro biodegradation measurement by viscosity method

The viscosity of the polymer solutions of the body fluid during degradation was measured using a Viscometer type (Grad-A-Ostwald) in a water bath with temperature (37°C). The intrinsic viscosity was calculated from the following equation [20,21]:

$$[\eta] = \frac{\sqrt{2(\eta_{sp} - \ln \eta_{rel})}}{C}$$

Where η_{rel} is the relative viscosity, η_{sp} is a specific viscosity; $[\eta]$ is the intrinsic viscosity, and C is the concentration.

3- Results and Discussion

FTIR Characterization of the prepared polymers

Poly(ethylene sebacate)

Polysebacoyl ethylene polymer, which was synthesized by condensation of glycol ethylene monomer with sebacoyl chloride, gave strong band at 3460 cm^{-1} which is corresponding to stretching of hydroxyl group of the terminal ethylene glycol monomer. Another band occurred at 1733 cm^{-1} that is characteristic of stretching frequency for carbonyl group of ester group, that result from reaction of the hydroxyl group of ethylene glycol monomer with sebacoyl monomer. Infrared bands occurred at 1242 cm^{-1} and 1282 cm^{-1} which are due to stretching frequencies of (C-O) group of ester as well. Another two strong bands occurred at 2700 cm^{-1} and 2887 cm^{-1} that attributed to stretching of aliphatic (C-H) group of methylene [22,23]. Infrared spectrum is shown in Figure (1).

Poly(diethylene sebacate)

This polymer gave band at 3461 cm^{-1} which is attributed to stretching of the hydroxyl group of terminal diethylene glycol monomer. Additional clear band occurred at 1735 cm^{-1} due to a carbonyl group stretching of ester group that formed due to condensation of the hydroxyl group of diethylene glycol monomer with sebacoyl chloride [23]. Another two bands occurred at 1176 cm^{-1} and at 1251 cm^{-1} which are due to stretching frequency of (C-O) group of ester group too, and another band in the range $(1041-1130)\text{ cm}^{-1}$ occurred attributed for stretching frequency of etheric (-C-O-C-) group. Two strong bands appeared at 2858 cm^{-1} and 2929 cm^{-1} which are due to stretching frequencies of aliphatic (C-H) group of methyl groups. Infrared spectrum is exhibited in Figure (2).

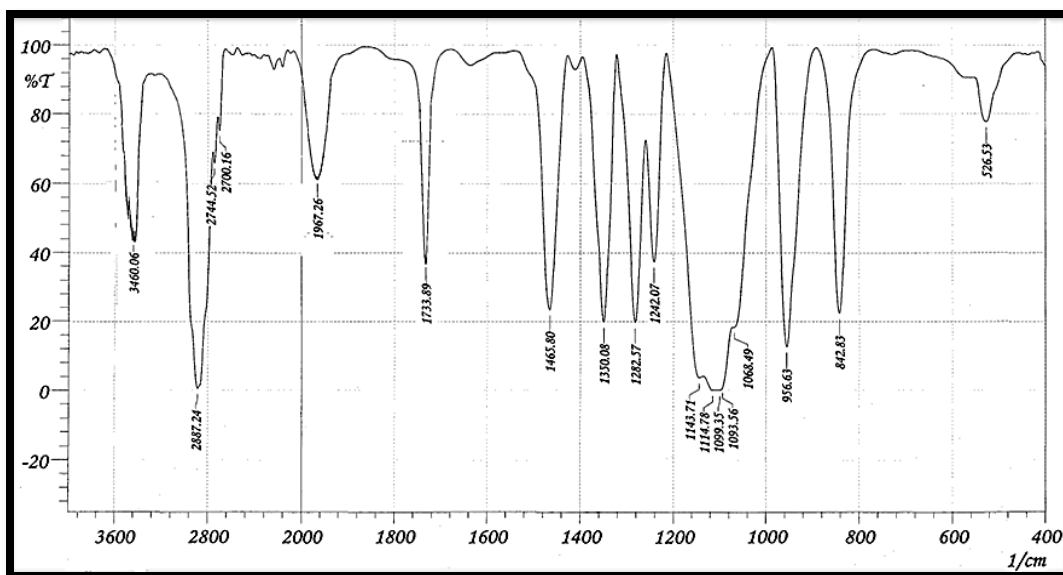


Figure (1): Infrared spectrum of poly(ethylene sebacate).

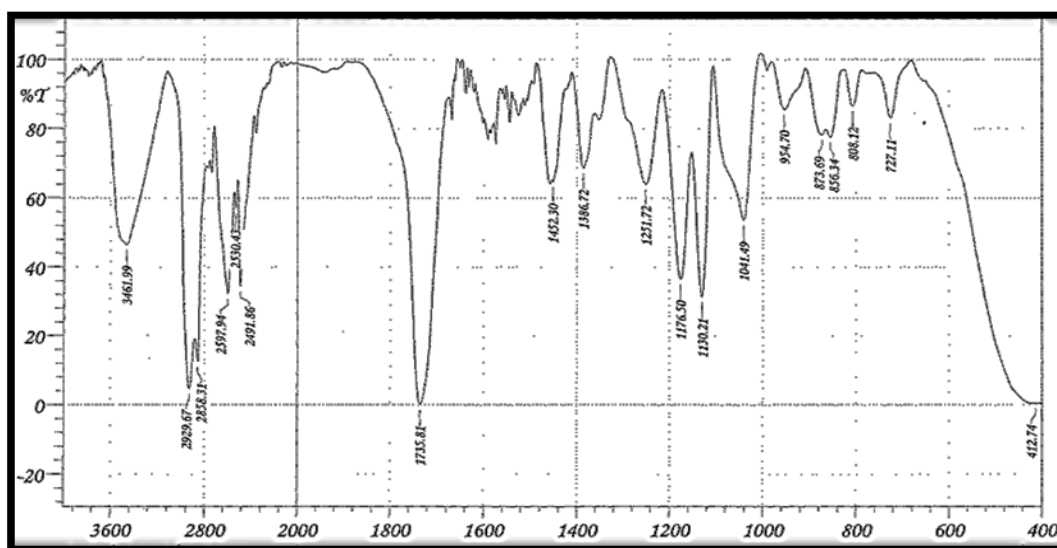


Figure (2): Infrared spectrum of poly(ethylene disbacate).

Poly(ethylene glycol)-Sebacate

Three different polymers were prepared having various molecular weight, 400, 10000 and 20000 g/mole by condensation polymerization of poly(ethylene glycol) with sebacoyl chloride. They were also characterized by FTIR.

Examining the FTIR spectra of poly(ethylene glycol)-sebacate showed broad bands in the rang ($3400\text{--}3600\text{ cm}^{-1}$) due to the stretching vibration of hydroxyl groups (O-H) which represent the alcoholic endings of polymer chains of poly(ethylene glycol) leaving the polyester functional

groups inside the structure, which they are appeared in the region (1735-1730 cm^{-1}). This may attributed to the presence of poly(ethylene glycol) to sebacoyl chloride in feed in 1.1:1 ratio. The important peaks and their assignments are listed in Table (2).

Table (4): The main absorption values poly(ethylene glycol)-sebacate prepared by different poly(ethylene glycol).

Stretching (cm^{-1})	Assigned bond	Functional group
3600-3400	O-H	-CH ₂ -OH
1735-1730	C=O	-CO-O-
1240-1150	C-O	-CO-O-
1120-1060	C-O	-C-O-C-
2900-2800	C-H	-CH ₂ -

Determination of the prepared polymers molecular weights

Number average molecular weights of the prepared polymers were determined using end-group analysis method [24]. Results obtained are shown in Table (3).

Table (3): Molecular weights of polymers prepared and number of the estimated repeated unit.

Polymer	M. Wt. of PEG used	M. Wt. of repeated unite (g/mole)	Determined polymer M. Wt. (g/mole)	No. of estimated repeated unit
Poly(ethylene sebacate)	-----	228	2260	10
Poly(diethylene sebacate)	-----	272	2300	8
Poly(ethylene glycol)-co-sebacate	400	566	3200	6
Poly(ethylene glycol)-co-sebacate	10000	10166	22600	2
Poly(ethylene glycol)-co-sebacate	20000	20166	23400	1

The molecular weights of polymers obtained from end-group analysis are listed in the Table (3), it was found that the number of repeated units of ethylene glycol in poly(ethylene sebacate) is slightly more than the number of repeated units of diethylene glycol in the poly(diethylene glycol) and this may be attributed to the reactivity of the first monomer over the diethylene glycol monomer [11]. This conclusion was quite clear in the preparation of poly(ethylene glycol)-sebacate produced from the condensation polymerization of poly(ethylene glycol) with sebacoyl chloride. It was found that the number of repeated units, i.e. poly(ethylene glycol), is affected by its chain length, nearly six repeated units are calculated using poly(ethylene glycol) having molecular weight 400 g/mole, Table (3), and start decrease to two repeated units as the molecular weight of poly(ethylene glycol) increases to 10000 g/mole. This may attribute to the difficulty of the polymerization process resulted from the chain movement disability stereoisomers, and this was fully supported by the only one repeated unit obtained using poly(ethylene glycol) with highest chain length used, 20000 g/mole of poly(ethylene glycol) [24].

Biodegradation of poly(ethylene sebacate) and poly(diethylene sebacate) by weight loss

Investigation of biodegradability of the poly(ethylene sebacate) and poly(diethylene sebacate) prepared by condensation polymerization of sebacoyl chloride with ethylene glycol and diethylene glycol; respectively, in weight loss method. The results obtained revealed that the poly(diethylene sebacate) polymer needed more than four months to reach only 21% loss and settled almost on this, while poly(ethylene sebacate) reached nearly 25% in the same period of time, as shown in Figures (3) and (4). This may attribute to their repeated unit chemical structure except that the presence of etheric linkage in diethylene glycol repeated units, giving rise more to non-crystallinity than the ethylene glycol repeated units.

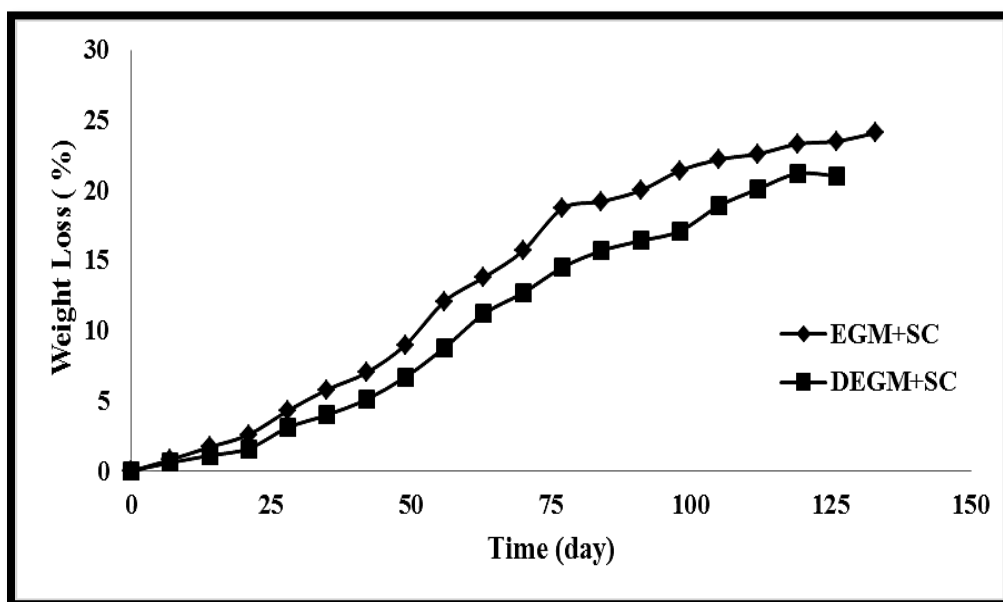


Figure (3): Effect of acid function (pH = 7.4) on the degradation of poly(ethylene sebacate) with time.

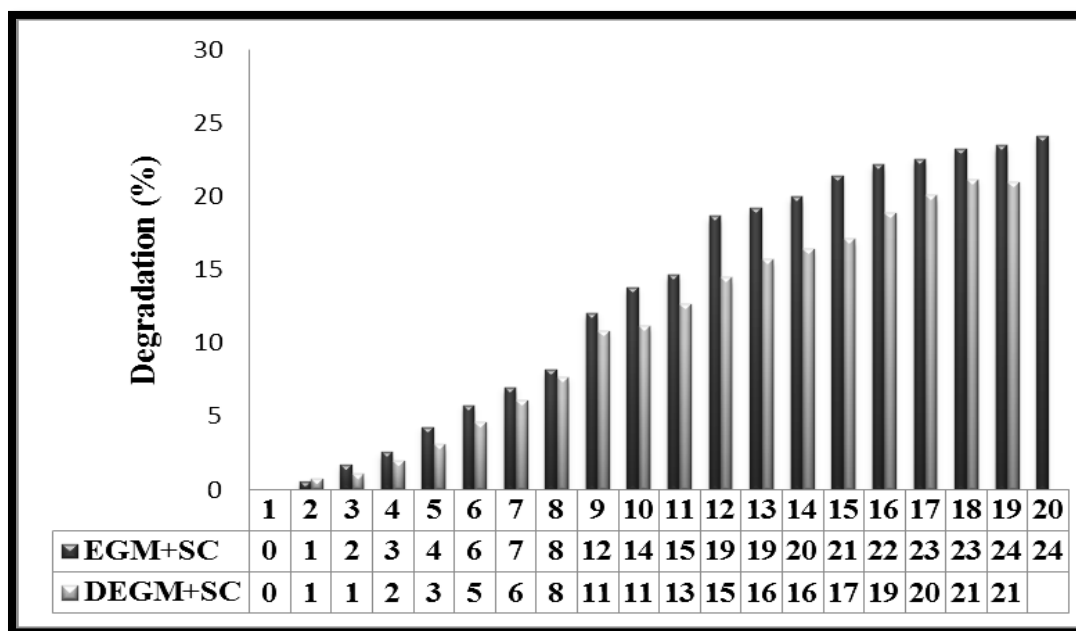


Figure (4): Percent degradation of poly(ethylene sebacate) and poly(ethylene disbacate) in acid function.

Biodegradation study by intrinsic viscosity measurement of prepared polymers

Intrinsic viscosities have been determined for periods of different degradation time under the same conditions used in weight loss measurement (pH = 7.4, 0.2g) and temperature (37 °C) [20,25].

Studying the degradation of the polymers prepared by measuring the intrinsic viscosity drop that indicates a decrease in molecular weight and the hydrolysis process occurs at pH = 7.4 with time, as shown in Figure (5) for both polymers, poly(ethylene sebacate) and poly(ethylene disbacate), following the same behavior in the %weight loss study.

Under the same conditions, degradation of the polymers produced from condensation polymerization of poly(ethylene glycol) having different molecular weight, i.e. (400, 10000, 20000) g/mole with sebacoyl chloride was also studied by determining of their degradable solution intrinsic viscosities. Figure (6) shows the recorded results. It is quite clear that the number of polymeric repeated units, and hence the polyethylene glycol chain length, has a great influence on the degradation of the polymer, the more repeated unit the less degradation. The same conclusion was reported in the literature [11,25].

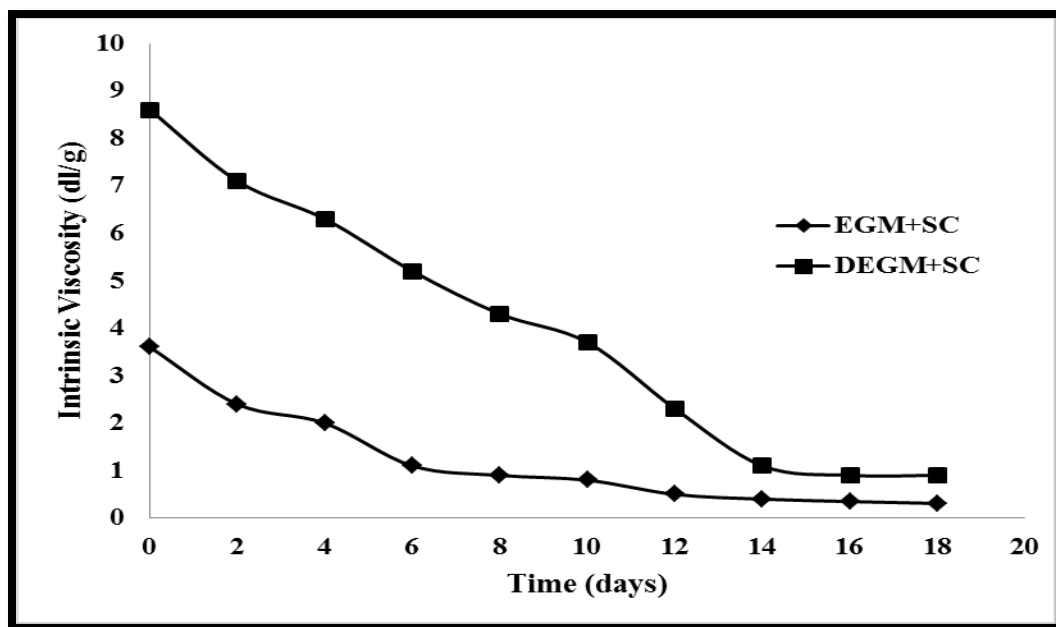


Figure (5): Influence of poly(ethylene sebacate) and poly(ethylene disbacate) biodegradation on their viscosity solutions.

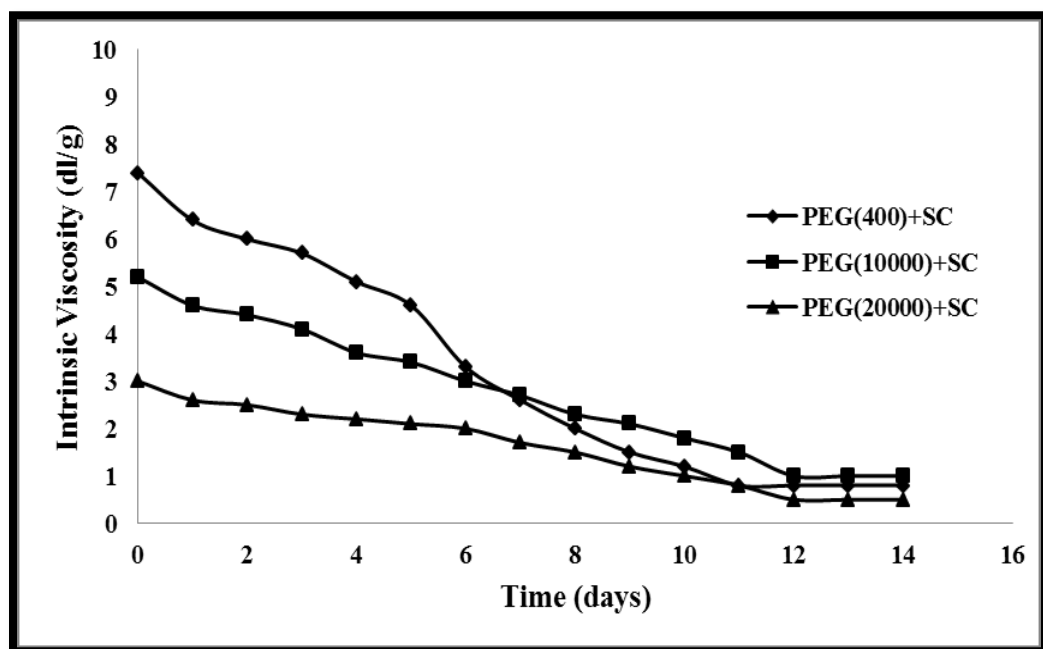


Figure (6): Effect of the number of repeated units on the intrinsic viscosity of the degradable polymer solution.

Conclusions

Polymers were prepared with high yield ranging from 81-88%, and this is more than that reported in the literature by (12-17%). Both poly(ethylene sebacate) and poly(diethylene sebacate) were resisted degradation as measured by % weight loss method, and the latter is better. This was attributed to the chemical structure of the diethylene glycol repeated units. The effect of chain length of poly(ethylene glycol) was also quite clear in the degradation of the polymer produced from their condensation polymerization with sebacoyl chloride as measured by intrinsic viscosity of their degradable solutions.

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دراسة التحلل الحيوي خارج الجسم الحي لبوليمرات محضرة من كلوريد السيباسويل مع
مونيمر (ثنائي) كلايكول الأثيلين وبولي (كلايكول الأثيلين)

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في هذه الدراسة، تم تحضير بعض بوليمرات كلايكول الأثيلين مع كلوريد السيباسويل بالبلورة التكتيفية وبالتحديد هي، بولي (سيباسات أثيلين) وبولي (سيباسات ثنائي الأثيلين) والبوليمر المشترك بولي (كلايكول الأثيلين) -سيباسات وبحصيلة عالية تراوحت بين 81-88%. جميع البوليمرات مصممة لتكون نهاياتها مجموعات هيدروكسيلية. شخّصت البوليمرات المحضرة بتقنية مطيافية الأشعة تحت الحمراء وتعيين معدل الوزن الجزيئي لها بطريقة تحليل نهاية-المجموعة، والطريقتين أكدوا صحة تراكيب البوليمرات المحضرة.

أستعمل في هذه الطريقة تشكيل البوليمرات على شكل اقراص وبوزن 0.2 غم وقطر 1سم ودراسة التحلل الحيوي لها خارج جسم الكائن الحي (*in vitro*) عند درجة حرارة الجسم (37°م) وبدالة حامضية (pH=7.4) والتي تمثل سائل الجسم. أستخدمت طريقتي فقدان الوزن وقياس اللزوجة الجوهرية لمحاليل البوليمرات خلال التحلل. كشفت النتائج أن بولي (سيباسات ثنائي الأثيلين) أحتاج الى أكثر من 4 أشهر ليصل الى نسبة 21% فقدان، بينما وصل بولي (سيباسات الأثيلين) الى 25% فقدان بنفس الفترة الزمنية. نفس السلوك لوحظ بقياسات اللزوجة.

وفي ظل الظروف نفسها، تمت دراسة تحلل البوليمرات الناتجة عن البلورة التكتيفية لبولي (كلايكول الأثيلين) ذات الوزن الجزيئي المختلف، أي (400، 10000، 20000) غرام/مول مع كلوريد السيباسويل، وذلك عن طريق تحديد حل قابلتم للتحلل وقياس اللزوجة الجوهرية لمحاليلها. النتائج التي تم الحصول عليها تعني أن عدد الوحدات البوليمرية المتكررة لها تأثير كبير على تدهور البوليمر، وأكثر بوليمر بوحدات متكررة أقلهم تدهور. ويعتبر هذا علامة جيدة لهذه البوليمرات لاستخدامها كناقل للأدوية في أنظمة تسليم الدواء المسيطر لأنها تزيد من وقت بقاء البوليمر المحمل بالدواء أطول فترة ممكنة في الجسم.