

Synthesis of aliphatic biodegradable polyesters nanoparticles as drug carrier for cancer treatment

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ABSTRACT

New amphiphilic triblock copolymers of poly(hexylene adipates) (PHA) and methoxy poly(ethylene glycol) (mPEG) having different hydrophobic/hydrophilic ratios were synthesized using a facile one-pot condensation reaction. Three samples of three different molecular weights were synthesized and characterized using different techniques. The synthesized copolymers were exploited to prepare core-shell nanoparticles with hydrophobic PHA and hydrophilic mPEG forming the core and shell, respectively. The drug loading efficiency and drug release properties of the mPEG-PHA-mPEG nanoparticles were investigated using 5-fluorouracil (5-FU) as a drug model. The drug loading efficacy of the nanoparticles was found to be dependent upon the polymer hydrophobicity. Drug release characteristics also depended on polymer hydrophobicity. The cytotoxicity of released 5-FU was monitored as an antiproliferative agent against human liver cancer cell line (HEPG2) as well as antimicrobial against both gram-positive and gram-negative bacteria. Results of the *in vitro* cytotoxic activity of the released 5-FU showed a sustained antiproliferative potency against the tested human liver cancer cell line for 26 days. Besides, results of the antimicrobial study revealed that the most potent inhibitory activity was against *S. aureus* which shows a hindrance zone of about 48 mm against the tested reference strain.

INTRODUCTION

Development of biodegradable nanoparticles for effective protein, DNA, drug, and peptide delivery is an interesting research area that has been attracting much attention in the past few decades (Barratt *et al.*, 2003). Loading of the drug onto a specially designed carrier has a lot of benefits, such as protection of the active substance against degradation *in vivo* and *in-vitro*, prolong its biological activity, the betterment of therapeutic effect, and control drug release rate (Couvreur *et al.*, 2006; Rawat *et al.*, 2013). One major downside of using nanoparticles as polymeric drug carriers is their rapid removal from the blood stream through phagocytosis after intravenous administration and recognition by the macrophages of the mononuclear phagocyte. Incorporation of the drug into a system

(Stolnik *et al.*, 19) keep the optimum level of the active constituent in the blood for extended time periods, long circulating polymer nanoparticles may be designed and utilized (Kataoka *et al.*, 2001; Torchilin *et al.*, 2002; Torchilin *et al.*, 1995). PEGylation is the almost frequent method used to prepare amphiphilic polymeric nanoparticles with different characteristics (Orive *et al.*, 2004; Owens *et al.*, 2006; Gryparis *et al.*, 2007). In this method, PEG is used as a hydrophilic segment that could be bonded to different hydrophobic segment. Examples of such hydrophobic segments are aliphatic polyesters. These polyesters are nowadays well known to be the most attractive and interesting materials to synthesize eco-friendly, biodegradable and biocompatible polymers (Dhamaniya *et al.*, 2010; Winzenburg *et al.*, 2004; Han *et al.*, 2004). Poly(lactide) (PLA), poly(ϵ -caprolactone) (PCL), poly(glycolide), (PGA), and poly (b-hydroxy butyrate) (PHB) are examples of such kind of polymers that have been potentially used as biodegradable and biocompatible polymers (Okada *et al.*, 2002).

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Another interesting category of the aliphatic polyester is those derived from polycondensation of di-carboxylic acids and dihydric alcohols, for examples, Poly(butylene succinate) (PBS) has been synthesized through condensation polymerization of succinic acid and 1,4-butanediol, while Poly(butylene succinate-co-adipate) (PBSA) is synthesized by polycondensation of succinic and adipic acids with 1,4-butanediol. Both have been commercialized under the trademark of BIONOLLE (Fujimaki *et al.*, 1998) and used as a green polymer to replace the traditional nondegradable plastics in many final products. Depending on the dicarboxylic acid and dihydric alcohol used to synthesize the polyester, their physical, mechanical, thermal and crystal structure properties are greatly influenced (Yang *et al.*, 2010; Ray *et al.*, 2005). 5-FU is one of the most extensively used agents in cancer therapy. Its active form inhibits DNA synthesis by inhibiting the usual production of thymidine. It has a comparatively high response in the colon, rectal, breast, gastrointestinal tract pancreas, head, ovarian cancers (Sastre *et al.*, 2007; Babu *et al.*, 2006; Wang *et al.*, 2005; Fournier *et al.*, 2003). There are many studies in the literature for encapsulation of 5-FU in polymeric materials (Helaly *et al.*, 2015; Helaly *et al.*, 2014; Menei *et al.*, 2004; Gudasi *et al.*, 2006). These polymers ultimately experience hydrolytic scission, producing by-products that can be metabolized in the body. Polymers like gel (Jeyanthi *et al.*, 1989), Chitosan (Huang *et al.*, 2010), co-poly(D,L-lactic/glycolic acid (Gupte *et al.*, 2004), poly(D-L-Lactide-co-glycolide) (Sastre *et al.*, 2007) have been exploited in the controlled delivery of 5-FU. The aim of the present work deals to:

1. Reproduce the method of Vassiliou *et al.* (2010) to prepare triblock copolymer nanoparticles from polyethylene glycol and polyhexylene adipate with different molecular weights (mPEG-PHA-mPEG) and applied as a carrier for the anticancer drug 5-Fluorouracil (5-FU). The targeted copolymers will be of type ABA, where the A is mPEG and represents the hydrophilic segment and B is polyhexylene adipate PHA that represents the hydrophobic segment. The polymers can be self-assembled forming shoe horse like micelles with a shell of mPEG and a core of PHA.
2. Study the sustained release of 5-FU in different pHs and determine the loading and release efficiencies of the drug.
3. Determine the cytotoxic activity of the released 5-fluorouracil against human liver cancer cell line as well as the antimicrobial potency against different bacteria strains namely *S. aureus*, *E. coli* and *C. albicans*.

EXPERIMENTAL

Materials

1,6-hexanediol was distilled before use (160 °C-undervacuum). Adipic acid and Triton X100 were purchased from MerckGermany. Methoxypolyethylene glycol mPEG (Mn 5000

g/mol), Titanium (IV) Butoxide was purchased from Sigma-Aldrich and used as received. 5-FU was purchased from Aldrich. All solvents are supplied from either Sigma-Aldrich or Merck and distilled before use.

Instrumentation and Characterization

Fourier transform infrared (FTIR) were recorded using a Jascow FTIR-430 (Japan) Instrument. Proton nuclear magnetic resonance (¹H NMR) was measured in CDCl₃ using Jeol ECA 500 MHz NMR spectrometer. Gel permeation chromatography (GPC) was measured using Agilent 1100 series-Germany, Detector: Refractive Index. Eluent is CHCl₃ using polyethylene oxide as a standard. High-resolution transmission electron microscopy images of polymeric nanoparticles were recorded by JEOL JEM 2100 T.E.M HR Japan. Thermogravimetric analysis (TGA) was conducted with Shimadzu TGA-50H. The measurements were performed at a heating rate of 10 °C min⁻¹ from 30 to 800 °C under anitrogen atmosphere. Differential scanning calorimetry analysis (DSC) was recorded on Shimadzu DSC-60 differential scanning calorimeter Columbia, EUA. All samples were heated/cooled with a scan rate of 10 °C/min over a temperature range of -100 to 100 °C under a nitrogen atmosphere. The particle size distribution of nanoparticles was determined by dynamic light scattering (DLS) using a Zeta-sizer Nano Instrument (Malvern Instruments, Nano ZS, ZEN3600, UK) operating with a 532 nm. The distribution curves of samples 4-6 are shown in Fig 5.

Synthesis of polyhexylene adipate (PHA) and (mPEG-PHA-b-mPEG) block copolymers.

A typical experiment of synthesis of PHA is as follow: 24.9 g (0.17 mol), of Adipic acid, 20.1 g (0.17 mol) of 1,6 hexanediol were mixed together in a pre-evacuated 1 L N₂-Flask under argon, then 0.05 mol % of titanium IV-butoxide was added via 1mL syringe. The system was connected to a condenser that ended with graduated Schlenk tube and placed in an oil bath at 170 °C. The reaction continued under N₂ until no more water was repelled out. At this point 0.1g % of Polyphosphoric acid was added via 1 mL syringe, N₂ supply was removed and vacuum (1.0mbar) was applied and the reaction left to run for 2 hours at 220 °C. At 0, 60 and 120 min of vacuum application, samples were withdrawn from the reaction vessel in order to obtain PHA with different molecular weights. The samples were then reacted at 220 °C for 1h under vacuum (1.0 Pa) with 3equiv. of mPEG of molecular weight 5000 g/mol using the same apparatus. The used amount of mPEG was calculated with respect to the carboxyl end groups of the used (Fras *et al.*, 2002). The obtained viscous polymer is left to cool down and dissolved in THF or chloroform and precipitated in methanol/n-hexane mixture 1/10. Dissolution/precipitation was repeated at least 3 times to remove the unreacted mPEG.

Preparation of drug-loaded nanoparticles

The drug-loaded polymeric nanoparticles were prepared by dissolving 500 mg of the copolymer and in 5 ml

dichloromethane and then added to 15 mL distilled water containing 0.1 mL triton X-100 to form O/W emulsion by using probe sonicator (300 watts, Sonic Vibro Cell, USA) for 5 minutes. During this process, 50 mg of 5-FU was added to the emulsion and again homogenized for 2 minutes. The formed O/W emulsion was stirred gently until the evaporation of the organic solvent was completed. The nanoparticles were collected by centrifugation (5000 rpm for 20 min).

The samples were reconstituted with deionized water. The suspension was filtered by a microfilter with apore size of 1.2 μm in order to remove polymer aggregates. To get the solid polymeric nanoparticles, the obtained micellar solutions were frozen and lyophilized in afreeze-drier. The weighed nanoparticles were dissolved in dichloromethane and the entrapped amount of 5-FU was determined by UV spectrophotometer (Agilent Tech., Cary series, wavelength 255 nm) and the nanoparticles yield, drug loading, and drug entrapment efficiency was calculated as described previously (Harisha *et al.*, 2010; Papadimitriou *et al.*, 2009).

$$\text{Nanoparticles yield (\%)} = \frac{\text{Weight of Nanoparticles}}{\text{weight of polymer and drug fed initially}} \times 100$$

$$\text{Drug loading (\%)} = \frac{\text{weight of drug in nanoparticles}}{\text{weight of nanoparticles}} \times 100$$

$$\text{Entrapment efficiency (\%)} = \frac{\text{weight of drug in nanoparticles}}{\text{weight of drug fed initially}} \times 100$$

The invitro drug release studies were performed using dialysis method. Weighed amount of drug loaded nanoparticles (20 mg) were dispersed in the release medium, (3 different media were used at pHs, 7.4, 8.5 and 4.1) and placed in a dialysis cellulose membrane bag (Fischer Scientific), having a molecular weight cut-off 6000-80000, tied and placed into a closed vessel containing the dissolution media. The dissolution media are phosphate buffer solution (0.1 M, pH 7.4), citrate buffer (20 mM pH 4.6) and borate buffer solution (pH 8.5). The whole system was placed in an incubator with anorbital shaker at 37 °C. At predetermined time intervals 3 ml of the aqueous solution was withdrawn from the release media and was replaced by fresh, medium. The samples were filtered and assayed for the drug by UV-Vis spectroscopy.

Anticancer testing

Measurement of potential cytotoxicity was done using SRB assay (Skehan *et al.*, 1990). The timely released 5-FU was

subjected to a screening system for evaluation of its antitumor activity against HepG2 liver cancer cell line.

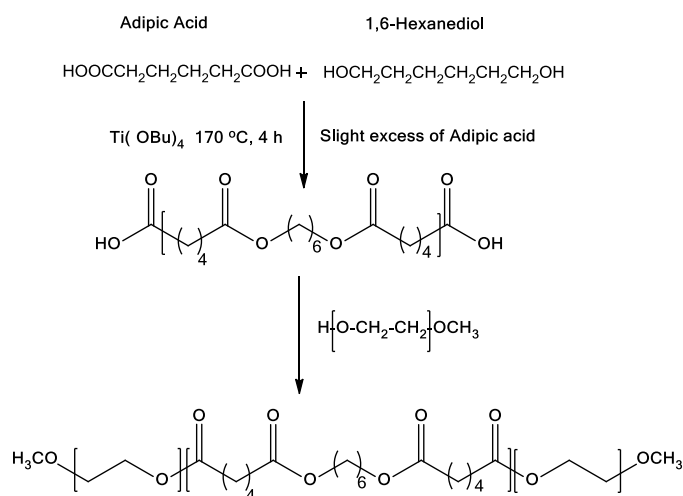
Antimicrobial testing

Antibacterial activity of the time intervals released 5-FU was in vitro evaluated using agar well diffusion test (Sgouras *et al.*, 2004; Wiart *et al.*, 2007) against the following tested stains; Gram-negative bacterial reference strain; *E. coli* O157 ATCC 700728, Gram-positive bacterial reference stains; *Strept. mutans* ATCC 25175, *Cl. perfringens* ATCC 13124 and Enterotoxigenic *S.aureus* ATCC 13565, mycotic reference strain; *C. albicans* EMCC 105 As control positive Antibiotic (AMC₃₀) (30 $\mu\text{g/ml}$) was used as standard antibacterial while Antimycotic (AMB₃₀) (30 $\mu\text{g/ml}$) was used as standard antifungi while dimethyl sulphoxide (DMSO) was used as control negative.

RESULTS AND DISCUSSION

Synthesis and characterization of the block copolymers.

The amphiphilic block copolymers (*mPEG-PHA-b-mPEG*) with different molecular weights were synthesized by polycondensation in one pot reaction over two steps. At first, carboxyl-terminated polyhexylene adipate (PHA) was obtained from the polycondensation of adipic acid and 1,6-hexanediol with very slight excess in the initial concentration of adipic acid in order to get carboxyl-terminated polyester. Samples were withdrawn at predetermined intervals to obtain PHA with different molecular weights. After that, polyethylene glycol monomethyl ether was condensed with the aforementioned samples for one hour under the same conditions to get three samples of the final block copolymer *mPEG-b-PHA-b-mPEG* (Scheme 1).



Scheme 1. Synthetic routeto synthesize *mPEG-b-PHA-b-mPEG*.

Molecular weights, polydispersity index, thermal analysis are presented in table (1). As can be seen from this table, the molecular weight of the homopolymers increases with the polymerization time. After only one hour of the condensation

reaction, the M_n was 8700 g/mol (sample 1, table 1). Further increase in the molecular weight was observed by continuing polycondensation when vacuum is applied at a higher temperature. The molecular weight was increased and jumped to be 15000 g/mol (sample 3, table 1). mPEG-PHA-mPEG triblock copolymers were synthesized by adding the monofunctional mPEG directly onto the melt polyester to condense with the carboxylic groups at both terminals.

Table 1: Molecular weight, conversion of the synthesized polymers.

Run	Time, h	M_n , g/mol	PDI	Conversion, %	mPEG (mol %)
Polyester homopolymers					
1	0.5	8700	3.04	n/a	n/a
2	1.0	10000	2.00	n/a	n/a
3	2.0	15000	1.97	n/a	n/a
Block Copolymers					
4	2.0	18000	2.02	87	77.5
5	2.0	19600	1.85	85	50
6	2.0	23500	2.16	85	35

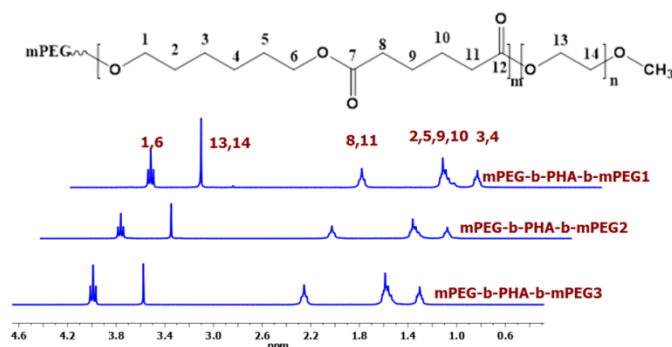


Fig. 1: ^1H NMR of mPEG-b-PHA-b-MPEG.

In this method, we have made the homopolymer PHA terminated with COOH groups to ensure the direct condensation with the less reactive high molecular weight mPEG. According to the M_n values, the transesterification reaction is minimized. This was agreed with the results of Gallardo and Pişkin (Gallardo *et al.*, 1998; Pişkin *et al.*, 1995). This signposts that mPEG, with a molecular weight of 5000 g/mol, was mostly added to the macromolecules. Fig.(1) represents the GPC elugram of both homo and block-copolymers. The absence of any shoulder at the neat mPEG positions ensure that there was no significant amount of unreacted mPEG remain after the purification process in the copolymers. Fig.2 shows the ^1H NMR spectrum of the prepared copolymers (mPEG-b-PHA-b-mPEG).

The peaks were assigned without any ambiguity according to that obtained by Kathrin *et al.* (Sun *et al.*, 2010). The peak at 3.65 ppm was assigned to the methylene protons of the mPEG block (13,14). The mPEG mole fraction in the polymers was calculated from the integrated peaks at (13,14) to those of at (1,6) ppm. It noted that the intensity of this methylene group of mPEG is decreased as the M_n of homo PHA is increased.

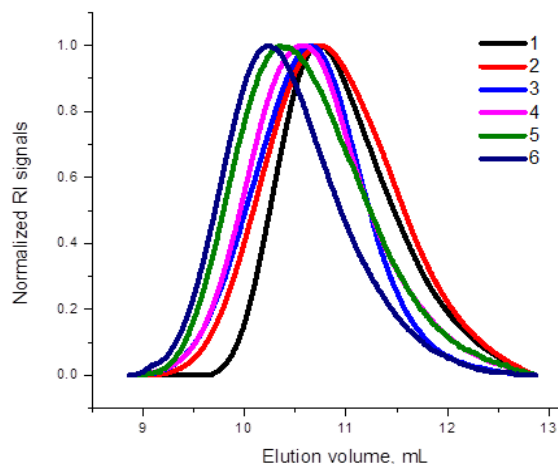


Fig. 2: GPC Elugrams of the synthesized polymers.

Thermal Characterization

The thermal stability of the synthesized block copolyesters was studied by DSC and TGA analysis and the curves are shown in Figs 3 and 4. The TGA curves in Fig 3 shows one-step degradation of the polymers. The polymers start to decompose at about 320 °C (0 % decomposition) indicating a high thermal stability of the whole block copolymer categories. Temperatures at 0 % and 5 % decomposition are listed in table 2.

Table 2: Decomposition temperature of homo and block copolymers at 0 and %5 decomposition.

sample	$T_{0\%}$, °C	$T_{5\%}$, °C
1	346	369
2	319	372
3	326	372
4	324	358
5	320	377
6	326	367

Thermal decomposition of linear aliphatic polyester gives at lower temperatures cyclic and linear esters or lactones, while at higher temperatures, further decomposition of the formed ester lead to give vinyl and carboxyl groups through ester scission and by cyclic elimination mechanism (Soccio *et al.*, 2007). Plage *et al.* reported that the chain length of the diol subunits of polyesters plays the main role in the thermal decomposition of polyesters while the impact of the dicarboxylic acid one is less important (Plage *et al.*, 1990). The melting behavior of the synthesized block copolyesters is studied using DSC measurements. The represented data in table 3, one can observe the melting point T_m of PHA homopolymers (samples 1 to 3) at 57 °C, glass transition temperature of -59 °C while the cold crystallization temperature T_{cc} appears at about 38 °C. The heat of fusion (ΔH_m) values for all homopolymer samples are in the same range and the same to the block copolymer samples. No noticeable outcome of the chain length (M_n) on all of these values. A slight lessening in melting point T_m from 57 to 54 °C in the case of block copolymers was observed. Because of the akin values of T_m of both mPEG and PHA, a reunion of the two peaks could be possible during the heating cycle (Fig 4). Tg values from PEG were not perceived but

it was reported in the literature at -57°C (Silva *et al.*, 2007) nearly as those of PHA and also could interfere with those of PHA segments as also shown by Ferrutti *et al.* and Cohen *et al.* (Ferrutti *et al.*, 1995; Cohen, *et al.*, 2000).

Table 3: Thermal data of the prepared block copolymers and homopolymers obtained from DSC measurements.

MPEG/ PE	mPEG segment				PHA segment			
	T _g °C	T _m °C	T _{cc} °C	Δ H _m J/g	T _g °C	T _m °C	T _{cc} °C	Δ H _m J/g
1	---	---	---	---	-59	57	37	81
2	---	---	---	---	-59	57	38	81
3	---	---	---	---	-60	58	38	81
4	---	---	16	---	-58	55	37	63
5	---	---	16	---	-56	54	36	65
6	---	---	17	---	-58	55	38	65

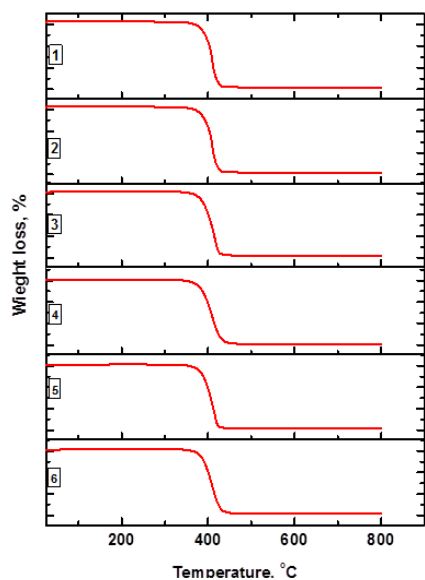


Fig. 3: TGA thermograms of the prepared block copolymers as well as the homopolymers

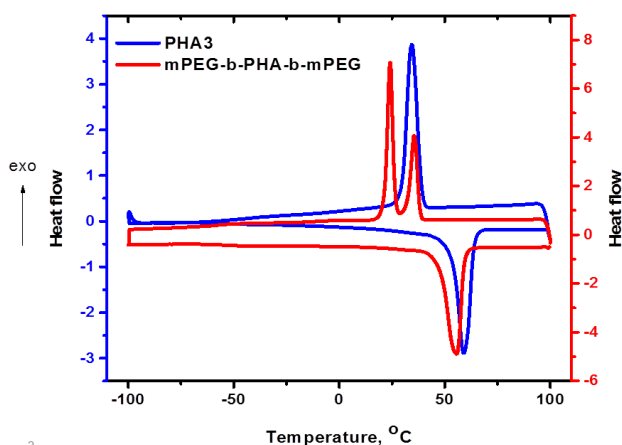


Fig. 4: DSC overlays of Heating and cooling cycles of PHA and mPEG-b-PHA-b-mPEG (runs 3, 6, table 3).

The DSC thermograms of both heating and cooling cycles of mPEG-PHA-b-mPEG (run 3 table 1) are shown in Fig 4, where the distinction between the two segments is observed in the

cooling cycle and T_{cc} of both segments could be detected easily. The occurrence of two melting T_{cc} points designates a kind of phase separation between the two segments during the cooling cycle, however, a merging of the two melting is seen during the heating cycle because both segments have comparable melting points.

TEM Characterization and size distribution of drug-loaded nanoparticles

Due to the amphiphilic nature, the prepared polymers can form micelles in aqueous media, where the hydrophobic segment PHA is forming the core of the micelles while the two mPEG segments form the tail and directed to the water medium to give shoe horse-shaped micelles as represented in Fig. (5) (He *et al.*, 2007). Table 4 shows the particle size distribution of the prepared drug-loaded mPEG-PHA-mPEG nanoparticles. The size of the nanoparticles varied between 164 to 190 nm with unimodal distribution, a small shoulder was appeared for the sample (6) with mean size of 530 nm but, it represents only 13 % of the whole sample as shown in Fig 6. These results are consistent with other copolymers, where the longer the hydrophobic block, the bigger the micelles (Hu *et al.*, 2007; Gaumet *et al.*, 2008; Chen *et al.*, 2006).

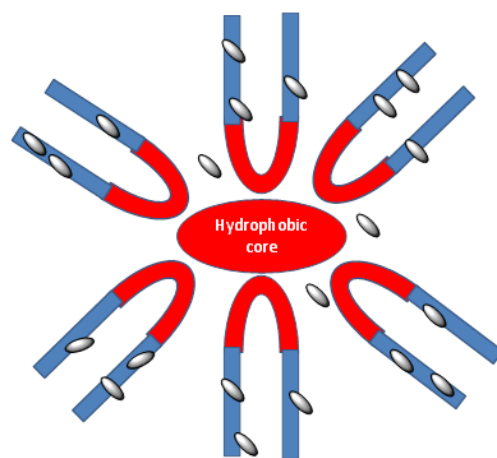


Fig. 5: A representative sketch showing the formation of mPEG-b-PHA-mPEG micelles

Table 4: Nanoparticle yield, drug loading content, entrapment efficiency, mean diameter and polydispersity index (PDI) of mPEG-b-PHA-mPEG nanoparticles loaded with 5-FU.

Sample	Mean diameter (nm)	PDI	Width nm	Drug loading (%)	Entrapment efficiency (%)	Nanoparticle yield (%)
4	164	0.151	87	7.3	54.8	60.5
5	164	0.145	77	7.9	53.4	61.1
6	190	0.277	67	8.6	51.6	61.3

A longer PHA block can boost its assembly to give larger core and, therefore, to a larger nanoparticle. Fig.6 shows representative TEM images of the prepared nanoparticles. All

images show that most of the drug-loaded nanoparticles had a clear spherical shape in aqueous media, with a mean diameter less than 200 nm, which is in agreement or even less with the results from the dynamic light scattering measurements. It is worthy to mention that the drug loading and drug entrapment efficiencies are slightly influenced by the length of the hydrophobic chain; there is a little increase in the drug loading capacity as the length of PHA increased. This may be referred to the corresponding increase in nanoparticle size.

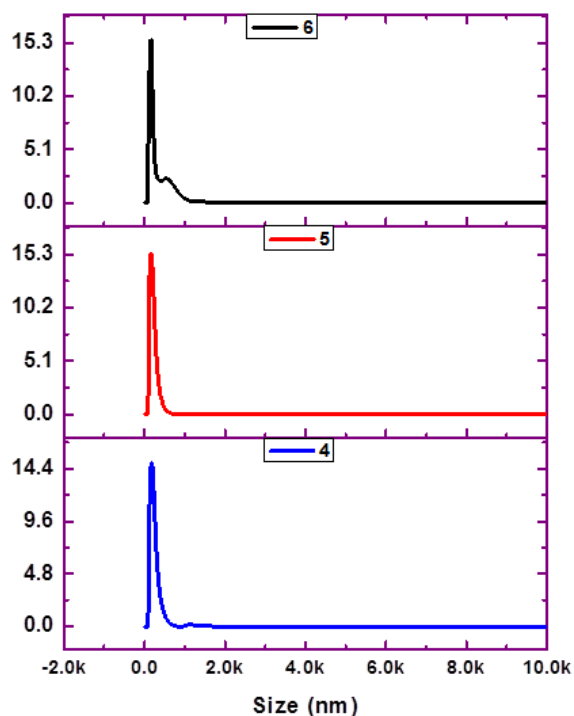


Fig. 6: Particle size distribution of the prepared nanoparticles.

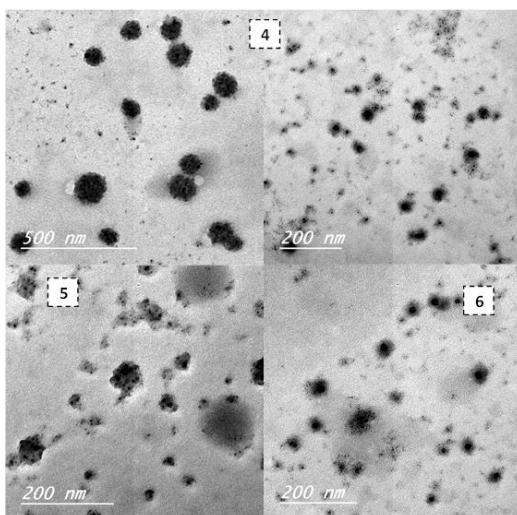


Fig. 7: TEM images of the prepared nanoparticles.

Drug release behavior

The release profiles of 5-FU from the prepared polymeric samples 4 to 6 in different pH media (7.4, 8.5, 4.6) are represented

in Figs. (8-11). A fast drug release was observed in the three media at early stages (until about 10 h.) with nearly the same rate for the three samples, only slight increase in the release rate in case of sample 4 at pH 7.4.

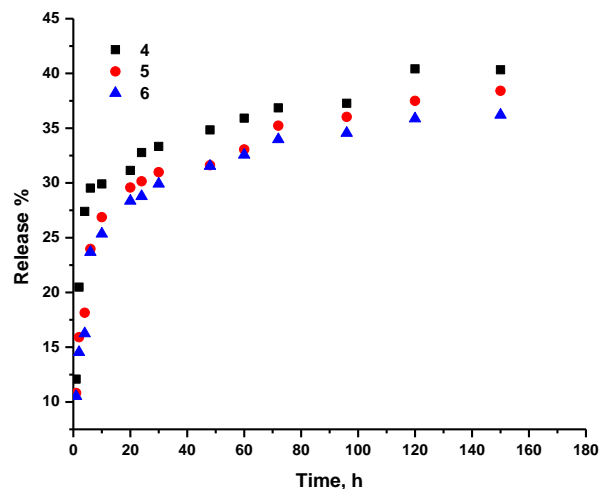


Fig. 8: Drug release profile for 5-FU from the prepared nanoparticles at pH 7.4

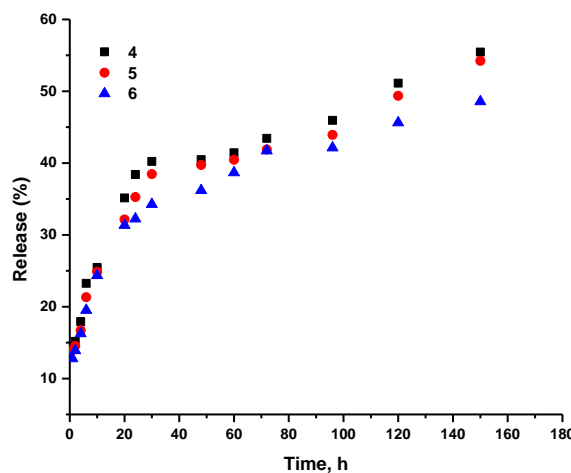


Fig. 9: Drug release profile for 5-FU from the three synthesized samples at pH 8.5.

As the release time goes further, the rate of the release goes slower at all pH media and for all molecular weights. It was also observed that the overall release rate of all samples was higher at pH (4.6 and 8.5), i.e. in both acidic and alkaline media. It was stated that more than one factor can influence the control release process of any drug from polymeric carriers. Instances of these factors are; degradation rate of the polymeric carriers, crystallinity, molecular weight, melting behavior, glass transition temperature, mode of crystallization, the ability of the drug to bind to the polymer, loading efficacy, etc. (Gref *et al.*, 1994; Zhang *et al.*, 2004). So the burst release at the beginning is expected to be due to a fraction of drug particles near to or on the surface of the

nanoparticles as also reported by Ge *et al.* (Ge *et al.*, 2002). Regarding the effect of pH on the release rate, it is also expected that the rate is increased in both acidic and alkaline media, due to the effect of hydrolysis of the hydrophobic moiety PHA leading to a fast release from the hydrolyzed residue (Assem, 2011) as shown in Fig.11. The effect of chain length on the release process is also observed. It is clearly seen from Figs 8-10 that sample 3 that possesses the lowest molecular weight is always giving a higher release rate than the others.

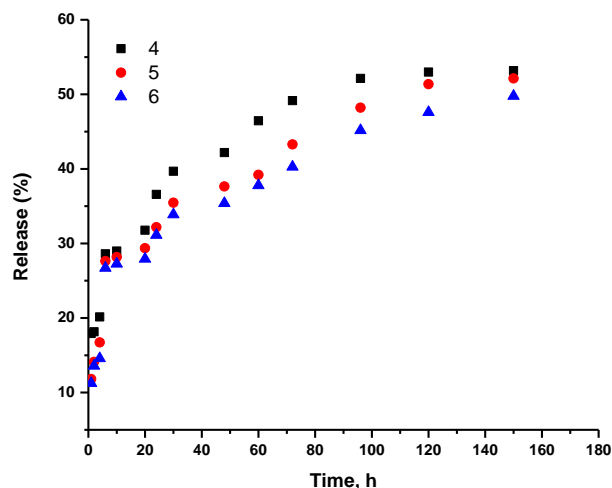


Fig. 10: Drug release profile for 5-FU from the three synthesized samples at pH 4.6

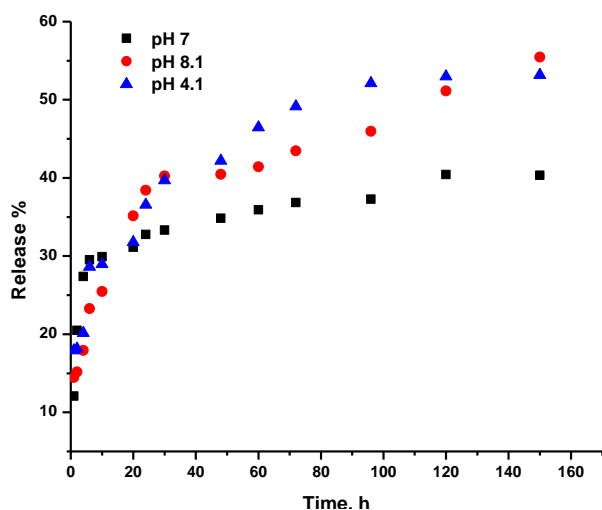


Fig. 11: Effect of pH on drug release rate for 5-FU from sample No 4 Table 1.

BIOLOGICAL RESULTS

Effect of released 5FU on Liver cancer cell line

It is interesting to evaluate the released 5-FU from the prepared polymeric nanoparticles on the human liver cancer cell line. Table (5) and Fig.(12) show the results obtained from the

practical experiment on human liver cell line HEPG2. It was found that promising results were obtained, the inhibition percent was extended to about 14 days.

Table 5: Single dose on HEPG2 cell-line (100µg/ml) 5-fluorouracil Released from mPEG-PHA-mPEG (Sample 4).

Released Samples	Concentration, mM	Time, h	Surviving, %	Inhibition, %
1	0.04	1	63.2	36.9
2	0.07	2	57.8	42.2
3	0.09	4	65.9	34.1
4	0.10	6	68.7	31.3
5	0.11	10	69.5	30.5
6	0.11	20	71.5	28.5
7	0.12	24	64.7	35.3
8	0.12	30	73.1	26.9
9	0.12	48	66.3	33.7
10	0.13	60	65.5	34.5
11	0.13	72	87.9	12.1
12	0.13	96	72.3	27.7
13	0.14	120	76.3	23.7
14	0.14	150	72.3	27.7

Antimicrobial effect of released 5FU on gram positive and gram negative bacteria

The released 5-FU was tested against different bacteria strains namely *S. aureus*, *E. coli* and *C. albicans*. Table 6 and Fig. 12 indicated that the best results were shown against *S. aureus* using the drug released from 1 to 14 which show great hindrance against the tested enterotoxigenic reference strain (*S. aureus*).

Table 6: Antimicrobial activity of 5- fluorouracil released from mPEG-b-PHA-mPEG (Sample 4).

Released Samples	Concentration ,mM	Time, h	Zone diameter,mm for		
			<i>E. coli</i>	<i>S.aureus</i>	<i>C. albicans</i>
PLATE 1					
1	0.0423	1	10S	34	-VE
2	0.0717	2	S	31	-VE
3	0.0959	4	13	33	-VE
4	0.1033	6	-VE	28	-VE
5	0.1047	10	15	30	-VE
6	0.1090	20	12	32	-VE
PLATE 2					
7	0.1147	24	14	34	-VE
8	0.1167	30	12S	34	-VE
9	0.1219	48	-VE	42	-VE
10	0.1257	60	12S	38	-VE
11	0.1290	72	-VE	-VE	-VE
12	0.1305	96	15	34	-VE
PLATE 3					
13	0.1415	120	16	33	-VE
14	0.1412	150	-VE	32	-VE
DMSO			-VE	-VE	-VE
ANTIBIOTIC(AMC ₃₀)			17	12	26
/ANTIMYCOTIC (AMB ₃₀)					

The best effect was no 9 and 10 with the zone of inhibition equals 42 and 38 mm, respectively, followed by release no 1,7, 8 and 12 with zone of inhibition equals 34mm then compounds 3, 13 and 15 with zone of inhibition 33mm then compounds 6 and 14 with the zone of inhibition equals 32mm and the least hindrance was with release 2 and 5 with the zone of inhibition 31 and 30 mm. The tested compounds were more effective against *S. aureus* is more than the reference drug AMC

30µg/ml which shows hindrance zone of 12-13mm against the tested ref strain. On the contrary, the tested compounds didn't show any hindrance abilities against the tested mycotic reference stain; *C. albicans* EMCC105. Although the reference drug AMB 30µg/ml gives azone of inhibition equals 26-30mm.

Few compounds were effective against tested reference *E. coli* O157 ATCC 700728as shown with samples no 13 and samples no5, 12 with the zone of inhibition equal 16mm and 15mm respectively. Followed by samples no 7,3 and 6 with the zone of inhibition equal 14,13 and 12 respectively. On the other hand, few compounds show the bacteriostatic effect of compounds no 8,10 and 1with zone of bacteriostatic equals 12 and 10mm, respectively.

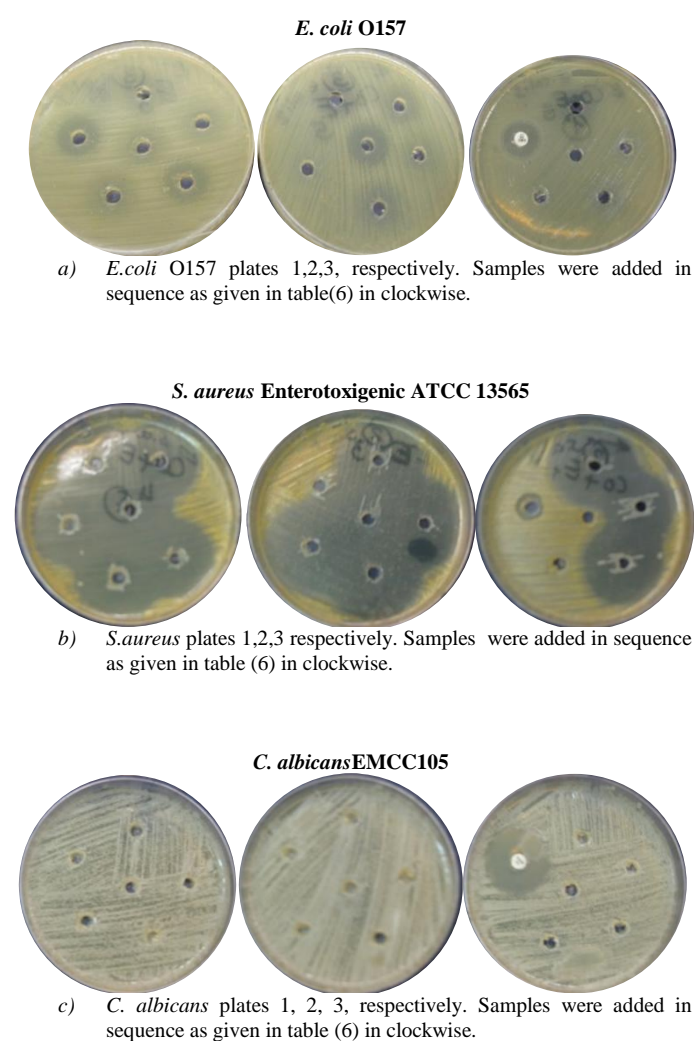


Fig. 12: Antimicrobial activity of 5- fluorouracil released from mPEG-b-PHA-mPEG against a) *E.coli*, b) *S.aureus* c) *C. albicans*

CONCLUSIONS

1. A degradable triblock copolymer of type ABA was successfully synthesized, where A represents the

hydrophilic segment that is methoxy-polyethylene glycol (mPEG) and B represents the hydrophobic segment that is polyhexylene adipate (PHA) and 3 different samples possessing different molecular weights are obtained.

2. Uni-modal nanoparticles were prepared from this block copolymer (mPEG-b-PHA-mPEG) using solvent displacement technique and used as a drug carrier for 5-FU.
3. Drug loading and entrapment efficiency were slightly influenced by the hydrophobic chain length.
4. A full drug release profile of 5-FU performed at different pHs and it was found to be higher in both acidic and alkaline media.
5. The sustained release of the active agent from the prepared copolymer showed that it can be a promising polymeric drug carrier for the antitumor drugs.
6. The cytotoxic activity of the released 5-fluorouracil indicated a sustained antiproliferative activity against the tested human liver cancer cell line as well as antimicrobial potency.

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