SYNTHESIS, CHARACTERIZATION AND CYTOTOXICITY OF CERTAIN PEG400 BASED BIODEGRADABLE ALIPHATIC COPOLYESTERS

B. Yamini^{1,*} and R. Nanthini²

¹Department of Chemistry, Saveetha Engineering College, Chennai, Tamilnadu, India

² Department of Chemistry, Pachaiyappa's College, Chennai-30, Tamilnadu, India

*E-mail: chemistryyamini@gmail.com

ABSTRACT

Random aliphatic copolymers synthesized by direct melt polycondensation reaction between Polyethylene glycol dodecanediol succinate (PEDDSU) and Polyethylene glycol dodecanediol sebacate (PEDDSE). This reaction occurred with titanium tetra-isopropoxide $Ti(ipo)_4$ as a catalyst. The characterization studies were examined by IR Spectroscopy, Nuclear magnetic resonance (NMR), Thermal analysis (DSC), Wide angle x-ray diffraction (WAXD) and molecular weight determination by Gel permeation chromatography (GPC). The viscosity and solubility studies are also measured for the newly synthesized copolyesters. Inherent viscosities were studied using ubbelohde viscometer. The addition of methylene units in the di-acid results in a decrease of T_m and T_g from the DSC analysis of copolyesters. The studies on number average and weight average molecular weight of these copolyesters have confirmed their molecular weights. We have also studied the anticancer activity of these copolyesters which were tested against *VERO* and *MCF-7* cell lines. The results of these studies conclude that both the obtained copolymers showed tremendous activity towards the anticarcinogenic property.

Keywords: aliphatic copolyester, polycondensation, differential scanning calorimetry, anticancer activity.

© RASAYAN. All rights reserved

INTRODUCTION

Aliphatic polyesters made from bifunctional acids and diols are expected to be competitive biodegradable polymers because they are easily digested and degraded completely by microorganisms¹. These copolyesters can easily enter the metabolic cycles of bio-organisms, readily susceptible to biological attack due to its non-toxicity^{2,3}. These copolyesters exhibit good solubility in most of the common solvents, essentially required for its applications^{4,5}. Previously with the absence of a catalyst, aliphatic polymers produced only low molecular weight polyesters derived from their respective diols and diacids. But with the presence of transesterification catalysts, high molecular weight polyesters are obtained with improved mechanical properties⁴. In general, high molecular weight polymers require a longer time to attain equilibrium^{6,7}.

Polyethylene glycol (PEG) is one of the polymers that is been widely accepted by food and drug administration (FDA), as the most biocompatible polymers for internal consumption^{8,9}. With PEG as a monomer, the properties like biocompatibility, hydrophilicity is improved and widely used as biomaterials¹⁰. Although the structure of PEG is very simple, its macromolecule causes cell fusion at very high concentration, also facilitates the movement of molecules across cell membranes. The hydroxyl groups available at both the ends provide chemically active sites to change the hydroxyls to other higher activity groups⁸.

Johnna S. Temenoff et al¹¹ designed to prepare a unique polymer oligo (polyethylene glycol fumarate) (OPF) to obtain hydrogels which possess swelling and mechanical property. Feixiong *et al*⁶ synthesized a series of monooleate poly (ethylene glycol) with one-ended active carboxylic terminal and succinic anhydride to complete the grafting of hydrophobic end-group oleate. Veeran Gowda Kaddajji et al¹² prepared water-soluble polymers for biomedical and pharmaceutical applications. Mayson H. Ziboon et

al¹³ synthesized poly (ethylene glycol)-sebacic acid with sebacic anhydride to produce biocompatible and nontoxic polymers.

In this proposal, the synthesis of aliphatic polyesters from 1, 12 - dodecanediol, PEG 400 and succinic / sebacic acid by melt polycondensation technique is described with respect to Titanium (IV) isopropoxide as the catalyst. The synthesized copolyesters are characterized by solubility, inherent viscosity, spectral studies, molecular weight determination and thermal analysis. The anticancer activities of these copolyesters and their cell viabilities were tested and compared against normal and adenocarcinoma cell lines.

EXPERIMENTAL

Material and Methods

PEG400, succinic acid, sebacic acid and 1, 12 - dodecanediol were purchased from Merck and used as such. Titanium (IV) isopropoxide purchased from spectrochem were used as a catalyst. Other chemicals and solvents were utilized directly.

Preparation of PEDDSU and PEDDSE copolymers

The synthesis of aliphatic copolyesters is carried out by two-step melt polycondensation method. The reaction proceeds with a three-necked round bottom flask furnished with dry nitrogen gas, an inlet tube, guard tube and magnetic stirrer purged with a mixture of PEG400, sebacic acid/succinic acid and 1, 12 - dodecanediol with a small amount of titanium (IV) isopropoxide as a catalyst. The reaction mixture is heated up to 160° C for 2 hours in the presence of nitrogen gas to remove the excess water. To attain post-polymerization, the temperature in the reaction flask is increased further to 200° C for 1 hour under reduced pressure to remove the traces of water. The obtained copolyester (*polyethylene glycol-co-dodecanediolsebacate*) and (*polyethylene glycol-co-dodecanediol succinate*) were purified by mixing with the smallest quantity of acetone; the mixture is vigorously stirred for 20 minutes with ice-cold methanol. The obtained slurry is washed with methanol, again and again, filtered and dried at room temperature under reduced pressure (760mm/Hg)⁴.

Instruments

The nuclear magnetic (¹H NMR) spectra were determined by Bruker AVIII 500 MHz with deuterated CDCl₃ as a solvent with TMS as the standard. Fourier transforms vibrational spectra of the copolyesters were recorded using potassium bromide pellets on a Shimadzu make over the range of 400-4000 cm⁻¹. In the Perkin Elmer DSC- 2 instrument, the values are scanned under a dry nitrogen atmosphere. The polymeric samples around 10 mg were weighed approximately and kept in a closed aluminium pan, scanned over a range of -50 °C to 300 °C and heated at a rate of 10°C/min. Thermal stability was determined by thermogravimetric analysis (TGA, Perkin Elmer TGS - 2 instruments) over the range of ambient to 800°C at a heating rate of 10 degree celsius per minute. The X-ray powder diffraction (WAXD) studies were conducted with a help of GE X-RAY 3003 diffractometer employing Cu K_α filtered radiation to inspect the amorphous / crystallinity of the polymer samples. The molecular weights

were determined on a waters GPC system (water 515 pumps) with RI detector 2414 at a temperature of 35 °C in the presence of THF as a solvent.

MTT Assay - Anticancer activity for Adenocarcinoma cell line

The antitumor activity of the normal and MCF-7 cell lines was resolved by the MTT assay (told by *Mossman et al.*, in the year 1983)¹⁴. The cells were seeded in 96-well plates (density: 10000 cells/well), incubated at 37°C with 5% CO₂ incubator. After confluence, the samples were expelled from the well and cleansed with phosphate-buffered saline (pH 7.4) solution. Then in a 100µl/well (5mg/ml) 0.5% of 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) were poured and incubated for 4 hours. The absorbance was measured with UV- Spectrophotometer at 540 nm using DMSO as blank. The results were calculated for both VERO and MCF- 7 cell lines. The viability percentage of the cells were calculated by using:

$$\text{Cell viability (\%)} = \text{A540 of viable cells} / \text{A540 of control cells} \times 100$$

RESULTS AND DISCUSSION

Solubility and Viscosity Measurements

The solubilities of the copolymers are displayed in the Table-1. The obtained copolyesters are insoluble in water, methanol, hexane and diethyl ether¹⁵. Inherent viscosities of the copolyesters were confirmed using chloroform as solvent at room temperature with the help of ubbelohde viscometer. The rate of flow time with respect to solvent and copolyester was found at a concentration of 1mg/ml¹⁶. The inherent viscosity of the copolyester PEDDSU is 0.3498 dL/g and PEDDSE is 0.3765 dl/g shown in the Table-1.

Table-1: Inherent viscosity and solubility of copolyester PEDDSU and PEDDSE

S. No.	Polymer	Inherent Viscosity dl /g	Freely Soluble	Sparingly Soluble	Insoluble
1	PEDDSU	0.3498	Chloroform, CCl ₄	Acetone	Water, Methanol
2	PEDDSE	0.3765	DCM & THF	DMF	Hexane, diethyl ether

Spectral Analysis

The FT-IR vibrational spectra were recorded in the transmission mode for the synthesized copolymers, shown in Fig.-1.

Fig.-1: FTIR Spectrum of PEDDSU and PEDDSE

The characteristic absorption bands for the synthesized copolymers are shown in Table-2.

Table-2: Absorption Range Frequency and its Assignment

Absorption frequency, cm^{-1}		Assignment
PEDDSU	PEDDSE	
1739	1727	C=O stretching of ester group
1153	1226	C-O stretching of ester group
1460	1469	Aliphatic C-C stretching
2926	2919	Aliphatic C-H stretching
3441	3440	Broad band assigned to hydroxyl group

¹H NMR Spectral Data of Copolyesters, PEDDSU, and PEDDSE

In the Proton NMR spectrum of PEDDSU, the multiplets split at 4.06 ppm to 4.09 ppm and 4.24 ppm correspond to the methylene protons attached to hydroxyl group^{17,18}. The signals at 3.63 ppm with four proton integrals were allocated to methylene protons attached to the carbonyl carbon of ester oxygen atom. In addition, the peaks obtained at 2.62 ppm to 2.66 ppm and 2.29 ppm to 2.34 ppm corresponds to the methylene protons of succinic and sebacic acid moiety. The other two multiplets observed at 1.26 ppm to 1.30 ppm and 1.60 ppm to 1.61 ppm was subjected to dodecanediol of the synthesized polymer^{19,20}. The copolyesters prepared from melt polycondensation have a random distribution of the monomers during the transesterification process^{21,22}. The spectra of polyesters are shown in Fig.-2 and 3 and the characteristic peak values of PEDDSU and PEDDSE are shown in Table-3.

Fig.-2: PEDDSU - ¹H NMR Spectrum

Table-3: ¹H NMR Spectral Data in ppm

PEDDSU (in ppm)	PEDDSE (in ppm)	Assignment
4.29, 4.06 – 4.09	4.22, 4.04 – 4.06	CH ₂ protons of the hydroxyl group
3.63	3.60 - 3.76	CH ₂ protons fixed to ester oxygen of carbonyl carbon atom.
2.62 – 2.66 & 2.29 – 2.34	2.27 – 2.34	Methylene protons attached to succinic / sebacic acid
1.26 – 1.30 & 1.60 – 1.61	1.27 – 1.30 & 1.61 – 1.62	Methy lene protons attached to dodecanediol

Fig.-3: PEDDSE - ^1H NMR Spectrum

The ^{13}C NMR spectrum was recorded to determine the skeletal structure of the copolymers with respect to deuterated chloroform as solvent.^{23,24} The peaks obtained in the range of 172.3 ppm to 172.4 ppm and 173.9 ppm to 177.6 ppm confirms the presence of ester carbonyl carbon²⁵. The other signals noticed from 69.0 ppm to 63.8 ppm and 70.9 ppm to 64.9 ppm was assigned to methylene carbons attached to hydroxy and ester groups. ^{13}C peaks detected from 25.8 ppm to 32.7 ppm were assigned to the rest of the methylene carbons in the acid and dodecane part of the compound. The signals viewed at 24.6 ppm to 29.4 ppm were due to the carbon atoms of succinic subunit²⁶. All the above characteristic peaks are shown in the Fig.-4 and 5.

Fig.-4: ^{13}C NMR Spectrum of PEDDSU

Fig.-5: ^{13}C NMR Spectrum of PEDDSE

From the confirmations of IR and NMR, we infer that the structural units in the copolymers are disbursed randomly.

Thermal Analysis

The thermal analysis is conducted for the synthesized copolymer to find their practical applications. The DSC thermograms of PEDDSU and PEDDSE are shown in Fig.- 6 & 7. The variation in the thermal behavior of the synthesized polymers depends on the increase in a number of methylene groups in the repeating unit. The glass transition temperature always determines the chain flexibility of the obtained polymers. From Fig.-6 and 7, it is observed that the T_g and T_m were found to be at -15.60°C and 145.76°C . Whereas, for PEDDSE the glass transition and melting temperatures were found to be at -75.0°C and 69.69°C . It was found that the glass transition and melting temperatures decrease with increasing number of methylene units. The lower the T_g , the more flexible are the polymer chains^{27,28}. The T_g and T_m values suggest that both the copolyesters can be used for drug delivery applications, which is partially matched with the temperature of human body¹⁵.

Fig.-8 and 9 show the TGA thermogram of PEDDSU and PEDDSE copolyesters. It can be seen from the graph that the decomposition temperatures (T_d) of the copolymers were observed at 358.70°C and 401.16°C for PEDDSU and PEDDSE. The decomposition temperature of sebacate copolymer is greater than succinate copolymer due to increase in chain length. A sharp bend noticed at 358.70°C and 401.16°C confirm its cristanallity. This result is also supported by wide-angle X-ray diffraction spectrum (WAXD)⁴.

X-ray Diffraction Analysis

The X-ray diffraction study is an effective tool to investigate the structure and cristanallity of the polymers. It is been performed for both the polymers PEDDSU and PEDDSE shown in Fig. 10 & 11 respectively. The figure below shows a broad reflection peak at $2\theta = 21.54^\circ$ and 21.4° which

determines the crystalline nature of the polymer .whereas the peaks pertaining at 24.81° , 41.55° , 43.71° , 23.79° and 40.43° indicates the formation of crystalline nature²⁹.

Fig.-6: DSC Spectrum of PEDDSU

Fig.-7: DSC Spectrum of PEDDSE

Fig.-8: TGA Spectrum of PEDDSU

Fig.-9: TGA Spectrum of PEDDSE

The increase in the length of flexible segments is indicated by the crystalline nature of the polymeric samples, which in turn increases the intensity of the diffraction peaks^{30,31}. From the values of x-ray diffraction measurement, it is perceived that the sebacate polymer is crystalline than the succinate polymer.

Fig.-10: XRD Spectrum of PEDDSU

Fig.-11: XRD Spectrum of PEDDSE

GPC Analysis

The relative molecular mass of the synthesized copolymers is conceived in Table-4. This technique determines the number-average and weight-average molecular weight of PEDDSU and PEDDSE. Reports reveal that this technique confirms the nature of copolyesters expected either in oligomeric or polymeric form³². The polydispersity index (M_w/M_n) and M_w of succinate copolyester are higher than sebacate copolyester conclude that the polymers were distributed in a random manner. And the lower molecular weight of the polymer is more suitable for biological application¹⁵.

Table-4: Molecular Weight for the Copolyesters PEDDSU and PEDDSE

S. No.	Polymer	M_w	M_n	DP	PDI
1	PEDDSU	4590	3841	11.9	1.19
2	PEDDSE	4215	3850	10.17	1.12

Antitumor Efficacy Studies

MTT Assay

The anticancer evaluation and its absorbance were identified by UV-spectrophotometer for the viable cells. The % cell viability and its IC_{50} values were determined for both *VERO* and *MCF-7* cells which are displayed in Tables -5, 6 and Tables -7, 8. By graphical determination, 50% inhibition of viability (IC_{50}) was calculated for both the copolyesters PEDDSU and PEDDSE which are shown from Fig.-12 to Fig.-19.

Table-5: Cytotoxic Values of Synthesized Compounds against *VERO* Cell Line in PEDDSU

Concentration Compound($\mu\text{g/mL}$)	Cell Viability (%)
1000	0
500	1.5
250	18.1
125	36.2
62.5	51.1
31.2	66.9
Control cells	100
IC 50 value	56.25 ($\mu\text{g/mL}$)

Fig. 12(a-d): Cytotoxicity effect of Compound PEDDSU on VERO cell line
a) Control; b) 1000 µg/mL; c) 62.5 µg/mL; d) 31.2 µg/mL

Fig. 13(a-d): Cytotoxicity effect of Compound PEDDSE on VERO cell line
a) Control; b) 1000 µg/mL; c) 62.5 µg/mL; d) 31.2 µg/mL

Fig. 14: Cell Viability of PEDDSU (VERO)

Fig. 15: Cell Viability of PEDDSE (VERO)

Table-6: Cytotoxic Values of Synthesized Compounds against VERO Cell Line in PEDDSE

Concentration (µg/ml)	Cell Viability (%)
1000	0
500	16.5
250	33.8
125	52.7
62.5	66.9
31.2	76.3
Control cells	100
IC 50 value	122.5 (µg/mL)

The PEDDSU and PEDDSE copolymers exhibited IC_{50} value (*VERO* Cell) at 56.25 $\mu\text{g/ml}$ and 122.5 $\mu\text{g/ml}$. but in the case of adenocarcinoma cells (*MCF-7*), the IC_{50} values were found to be 31.08 $\mu\text{g/ml}$ and 29.77 $\mu\text{g/ml}$. From the results of *VERO* cell lines it is inferred that PEDDSE is more cytotoxic than PEDDSU due to higher IC_{50} values. But from the results of *MCF-7* cells, sebacate copolymer showed more anticancerous property than succinate copolymer due to lower (IC_{50}) values³³. The maximum cancerous cell death occurred in sebacate when compared with succinate. Therefore from these results we conclude that PEDDSE is antiproliferative than PEDDSU^{34,35}.

Table-7: Cytotoxicity of Synthesized Compounds against *MCF-7* Cell Line in PEDDSU

Concentration Compound($\mu\text{g/ml}$)	Cell Viability (%)
1000	19.06
500	22.95
250	33.85
125	41.43
62.5	47.85
31.2	55.25
15.6	60.70
7.8	69.45
Cell control	100
IC_{50} value	31.08 ($\mu\text{g/ml}$)

Table-8: Cytotoxicity of Synthesized Compounds against *MCF-7* Cell Line in PEDDSE

Concentration ($\mu\text{g/ml}$)	Cell Viability (%)
1000	15.38
500	23.80
250	32.05
125	36.85
62.5	42.99
31.2	52.39
15.6	57.96
7.8	62.76
Cell control	100
IC_{50} value	29.77 ($\mu\text{g/ml}$)

Fig. 16(a-d): Cytotoxicity effect of Compound PEDDSU on MCF-7 cell line: a) Control ; b. 1000 $\mu\text{g/mL}$; c. 15.62 $\mu\text{g/mL}$; d. 7.8 $\mu\text{g/mL}$. Fig. 17(a-d): Cytotoxicity effect of Compound PEDDSE on MCF-7 cell line: a) Control ; b. 1000 $\mu\text{g/mL}$; c. 15.62 $\mu\text{g/mL}$; d. 7.8 $\mu\text{g/mL}$.

Fig. 18: Cell Viability of PEDDSU

Fig. 19: Cell Viability of PEDDSE

CONCLUSION

Aliphatic copolyesters were synthesized from PEG400, succinic acid, sebacic acid and 1, 12 - dodecanediol by a simple, safe and cost-effective melt poly condensation technique. The copolyester displays great dissolvability in familiar solvents which is an essential quality in perspective of its applications. The resulting structures of the repeating units were authorized on the basis of nuclear magnetic resonance spectrum. The thermal properties outlined by the differential scanning calorimetry and TGA analysis recorded that the T_g and T_m decreases with increasing number of methylene units. The thermal stability also confirms the decomposition temperatures were at 358.70° C and 401.16° C, widely used in drug delivery applications. From the XRD studies, it is exquisite that PEDDSE is crystalline than PEDDSU. From GPC analysis, the molecular weights were identified for sebacate and succinate copolyester. The antitumor efficacy studies show that the PEDDSE copolyester is more anticarcinogenic than PEDDSU.

ACKNOWLEDGEMENT

The authors thank the Central Leather Research Institute (CLRI), Chennai and Indian Institute of Science (IISC), Bangalore for providing thermal analysis and GPC studies.

REFERENCES

1. S. Soulis, D. Triantou, S. Weidner, J. Falkenhagen and J. Simitzis, *Polymer Degradation and Stability*, **97**, 2091 (2012), DOI:10.1016/j.polyimdegradstab.2012.09.002.
2. P. Rizzareli, G. Impallomeni and G. Montaudo, *Biomacromolecules*, **5**, 433 (2004), DOI: 10.1021/bm034230s.
3. A. Lindstrom, A.C. Albertsson and M. Hakkarainen, *Polym Degrad Stab*, **83**, 487 (2004), DOI: 10.1016/j.polyimdegradstab.2003.07.001.
4. S. S. Umare, A.S. Chandure and R.A. Pandey, *Polymer Degradation and Stability*, **92**, 464 (2007), DOI:10.1016/j.polyimdegradstab.2006.10.007 4.
5. S. M. Nikolic, D. Poleti and J. Djonlagic, *European Polymer Journal*, **39**, 2183 (2003), DOI:10.1016/S0014-3057(03)00139-3.
6. F. Xiong, J. Li, H. Wang, Y. Chen, J. Cheng and J. Zhu, *Polymer*, **47**, 6636 (2006), DOI:10.1016/j.polymer.2006.07.020.
7. Z. H. Kang and C. L. Wang, *Advanced Materials Research*, **750**, 1313 (2013), DOI: 10.4028.
8. J. M. Harris, *Poly (Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications*, Plenum Publishing, New York, 1992
9. A.S. Chandure, *European Polymer Journal*, **44**, 2068 (2008), DOI: 10.1016/j.eurpolymj.2008.01.001.
10. Q. Liu, L. Jiang, R. Shi and L. Zhang, *Progress in Polymer Science*, **37**, 715 (2012), DOI: 10.1021/bm0343242.

11. S. Johnna, A. T. Kyriacos, Athanasiou, G. Richard, L. Antonios and G. Mikos, *Journal of Biomedical Materials Research*, **59**, 429 (2002), DOI:10.1002/jbm.1259.
12. V. G. Kadajji and V. G. Betageri, *Polymers*, **3**, 1972 (2011), DOI: 10.3390/polym3041972.
13. H. M. Ziboon, A. A. Saleh and S. H. Al-Lami, *International Journal of Advanced Research*, **2**, 159 (2014).
14. T. Mosmann, *J. Immunol.Methods*, **65**, 55 (1983).
15. L. Sowbagyalakshmi Prabha, R. Nanthini and G. Krishnaveni, *Journal of Chemical and Pharmaceutical Research*, **4**, 2442 (2012).
16. J. Gowsika and R. Nanthini, *Journal of Chemistry*, **2014**, 1 (2014), DOI:10.1155/2014/173814.
17. W. Dong, J. Ren, L. Lin, D. Shi, Z. Ni and M. Chen, *Polymer Degradation and Stability*, **97**, 578 (2012), DOI:10.1016/j.polymdegradstab.2012.01.008.
18. P. S. Achary, *The Journal of Adhesion*, **78**, 695 (2002), DOI:10.1080/00218460213492.
19. J. Ling, W. Chen and Z. Shen, *Journal of Polymer Science*, **43**, 1787 (2005), DOI:10.1002/pola.20648.
20. A. S. Chandure, *International Journal of Polymeric Materials*, **56**, 339 (2007), DOI: 10.1080/00914030600865093.
21. L. Sowbagyalakshmi Prabha, R. Nanthini and K. Prabhu, *Oriental Journal of Chemistry*, **28**, 1659 (2012), DOI:10.13005/ojc/280416.
22. U. C. Mashelkar, J. B. Patil, R. S. Kenny and N. R. Chindarkar, *Rasayan. J. Chem.*, **8**, 422 (2015).
23. M. Mazurek, T. Bruliński, K. Tomczyk, P. Parzuchowski, Z. Florjańczyk, A. Plichta and G. Rokicki, *Journal of Polymer Research*, **34**, 1 (2015), DOI: 10.1007/s10965-015-0655-0.
24. O. Ahmed and H. El-Nezhawy. *Archiv der Pharmazie*, **344**, 631 (2011), DOI:10.1002/ardp.201000367.
25. T. Kamalanathan and R. Nanthini, *Universal Journal of Pharmacy*, **02**, 102 (2013).
26. M. Soccia, N. Lotti, M. Gazzano, M. Govoni, E. Giordano and A. Munari, *Reactive and Functional Polymers*, **72**, 856 (2012), DOI:10.1016/j.reactfunctpolym.2012.08.002.
27. V. Karavelidis, D. Giliopoulos, E. Karavas and D. Bikiaris, *European Journal of Pharmaceutical Sciences*, **41**, 636 (2010), DOI:10.1016/j.ejps.2010.09.004.
28. P. Rizzarelli, C. Puglisi and G. Montaudo, *Polymer Degradation and Stability*, **85**, 855 (2004), DOI:10.1016/j.polymdegradstab.2004.03.022.
29. A. Ravi, R. Nanthini, M. Karunanidhi and V. Jaishankar, *Oriental Journal of Chemistry*, **26**, 547 (2010).
30. S. Papadimitriou, D. Bikiaris, *Journal of Controlled Release*, **138**, 177 (2009), DOI:10.1016/j.jconrel.2009.05.013.
31. Y. Chen, Y. Yang, J. Su, L. Tan and Y. Wang, *Reactive and Functional Polymers*, **67**, 396 (2007), DOI:10.1016/j.
32. F. T. Tan, G. D. Cooper, M. Maric and A. J. Nicell, *Polymer Degradation and Stability*, **93**, 1479 (2008), DOI:10.1016/j.polymdegradstab.2008.05.005.
33. R. B. Badisa, O. Tzakou, M. Couladis and E. Pilarinou, *Pharmaceutical Biology*, **42**, 640 (2005), DOI: 10.1080/13880200490902590.
34. J. Kaban, J. Reveny, J. Tarigan and N.F. Zebua, *Rasayan. J. Chem.*, **11(1)**, 294 (2018).
35. V. N. Ariharan and P. N. Prasad, *Rasayan. J. Chem.*, **7**, 260 (2014).

[RJC-1992/2017]