Journal of Science and Technology **54** (5A) (2016) 45-55

D,L-SERINE BASED pH-SENSITIVE OLIGOESTER

Dang Nguyen Tri[1], Dai Phu Huynh[1, 2, *]

[1]*Faculty of Material Technology, Ho Chi Minh University of Technology, HCMUT–VNUHCM, 268 Ly Thuong Kiet Street, Ward 14, District 10, Ho Chi Minh City*

[2]*National Key Laboratory of Polymer and Composite Materials, HCMUT–VNUHCM, 268 Ly Thuong Kiet Street, Ward 14, District 10, Ho Chi Minh City*

[*]Email: *hdphu@hcmut.edu.vn*

Received: 15 July 2016; Accepted for publication: 3 December 2016

ABSTRACT

In recent years, pH/temperature-sensitive polymers have attracted increasing attention as drug/protein delivery systems. In this study, the main objective was to synthesize a pH-sensitive oligoester. The oligoester was synthesized by condensation reaction from carboxylic and hydroxyl groups of D,L-Serine, which had been previously modified with benezenesulfonyl chloride in order to create sulfonamides as pH sensitive groups. Various molecular weights of the oligoesters were obtained by means of manipulating the mole ratio of N,N'-Dicyclohexylcarbodiimide (DCC)/Serine (DCC acts as a coupling agent) and the Dimethylformamide (DMF)/Serine (v/w) ratio (DMF acts as solvent). The synthesized oligoesters were characterized by ^{1}H-NMR and their molecular weights were measured by gel permeation chromatography (GPC). Also, the pK_a values of pH-sensitive oligoesters were obtained by the titration method. This pH dependent property of the polymers could be very useful for preparing drug carriers that are sensitive to pH environment.

Keywords. pH sensitive, D,L-Serine, benezenesulfonylchloride.

1. INTRODUCTION

In recent years, stimuli sensitive polymers have attracted the interest of researchers for their applications in drug delivery systems and protein [1, 2]. There are many sensitive factors of environmental stimuli such as temperature, pH, light, electric field, and other stimuli [2, 3 - 7]. In particular, in the biomedicine field, throughout years, there are many studies focusing on the temperature-sensitive polymer capable of biodegradation because of their advantages for drug delivery systems like easily-controlled molecular weight, very biocompatible, ... It is aimed to be a drug carrier or an implant, ... Recent decades, the temperature-sensitive polymer blocks have been applied in drug transfer as an injectable hydrogel. However the temperature-sensitive polymer hydrogels have some limitations: the transformation of polymer solution into gel in the needle during the injection into the body due to the heat transfer from the body to the syringe. That is a difficulty for the injection [8 - 10]; when injected into the body, the hydrogel tends to

degrade to form monomers, which have the potential to reduce the ability to load and release drugs [1].

Hence, there is a need to create a responsive polymer hydrogel, which can respond to more than a stimulus to solve this problem and the pH-sensitive polymer is candidate that researchers have used to overcome the drawbacks of temperature-sensitive hydrogel. Because, besides temperature, pH is an important environmental factor for drug delivery system for living body, it changes in many identified organs such as: stomach, intestine, intracellular organelles pepper can, vascular, vagina, and the area of the tumor. The ionizable groups in the polymer (base functional groups and acid functional groups) are pH sensitive agents, they act as the hydrophilic or hydrophobic portion of the polymer based on the ionizing and de-ionizing ability of pH-sensitive polymers. Then, the process of sol-gel transition is seen as a change of the hydrophobic property of polymer [9, 10]. Therefore, pH-sensitive polymers in pharmaceutical applications have attracted the attention of researchers.

In this study, we used D,L-Serine, whose amino groups had been previously modified with benezenesulfonyl chloride in order to create sulfonamides, as a raw material for the synthesis of oligoester having pH-sensitivity. The oligoesters were synthesized by the method of condensation reaction. The sulfonamide groups in oligoesters are capable of ionizing. The condensation reaction in solution is a new approach in a pH-sensitive polymer synthesis applied in drug delivery systems. In this research, we surveyed the influence of elements ratio (D,L-Serine, DCC, DMF) to the molecular weight of the oligoesters to obtain pH sensitive oligomers as already set targets. Moreover, D,L-Serine is a natural substance and has been widely used in food and biomedicine as spices and minerals for human body. Therefore, D,L-Serine could be very useful for preparing drug carriers.

2. EXPERIMENTAL

2.1. Materials

D,L-Serine was bought from Sigma-Aldrich (purity of 99.99 %). 4-Dimethylaminopyridine (DMAP) and 1,3-Dicyclohexylcarbodiimide (DCC) were purchased from Sigma-Aldrich, they were used as a catalyst system of polymer condensation. All other reagents were of analytical grade and used without further purification.

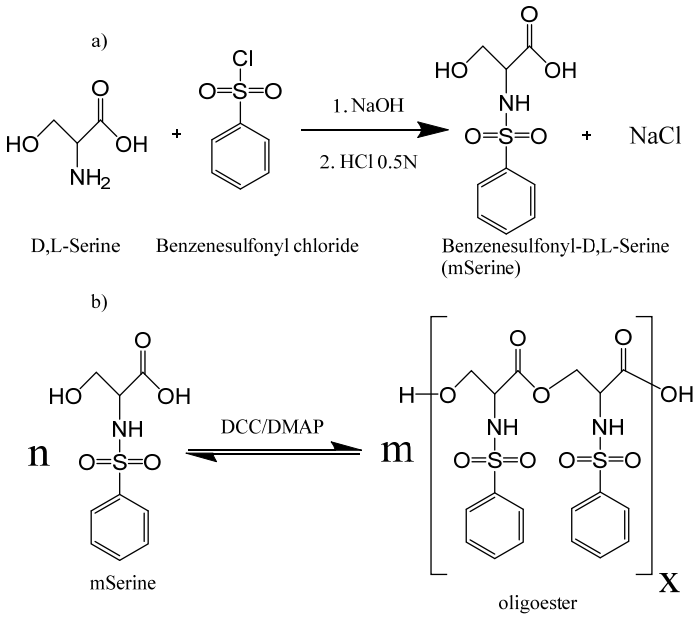
2.2. Methods

2.2.1. Modification of D,L-Serine with Benzenesulfonyl chloride

NaOH solution was first prepared by adding 3.04 g NaOH in 60ml H_2O, D,L-Serine (4.0 g) was then poured into this solution at 0 °C. After stirring for 2h, Benzenesulfonyl chloride (4 ml in 40 ml 1,4-Dioxane) was added dropwise, and the reaction took place at 25 °C for the next 10 h. As soon as the reaction was accomplished, the reaction solution was washed by diethyl ether with an excess amount. After that, the organic layer was completely removed while the aqueous was acidified with 10%HCl to a pH 1-2 and then extracted with ethyl acetate. In the next step, $MgSO_4$ (drying agent) was used to absorb water residue in the ethyl acetate layer. $MgSO_4$ were then filtered out of these layers by paper filters (0.2 mm), and the organic solution was evaporated under vacuum to give Benzenesulfonyl-D,L-Serine (mSerine) (Scheme 1).

2.2.2. Synthesis of oligoesters

To start with, a predetermined amount of mSerine was dissolved in DMF at room temperature. The feed ratios of DCC/Serine (mole/mole) and DMF/Serine (v/w) varied. The reaction system was carried out in Argon environment. In the next step, the catalyst system (DCC/DMAP) was weighed and injected into the reaction solution. After 1h' reaction, DCU (byproduct) was completely removed from the reaction solution by PTFE filter (0.45 μm). DMF was then eliminated via a rotary evaporation at 80 °C, and the dried residue was dissolved in chloroform. Next, the crude product was purified by precipitation into excess diethyl ether in order to obtain oligoesters (OS). The product was dried under vacuum at 60 °C for 48 h (Scheme 1).



Scheme 1. Synthesis of oligoester based on D,L-Serine a) Synthesis of pH-sensitive monomer (mSerine). b) Synthesis of oligoester.

2.2.3 ^{1}H NMR Analysis

The ^{1}H-NMR spectrums were obtained from Bruker Avance machine at 500MHz and used to determine the molecular structures of D,L-Serine and oligoesters. D_2O was used as solvent for glycine and spectrum of OS was analysed in DMSO solvent containing 0.03 % (v/v) Tetramethylsilane (TMS).

2.2.4. GPC Analysis

The molecular weight and molecular weight distribution of oligoesters were measured by gel permeation chromatography (GPC) Agilent 1100 Series, with a PLgel MIXED column, at 50 ^{0}C. DMSO was used as an eluent at a flow rate of 1 mL/min. Calibration was carried out using poly(ethylene glycol) (Polymer laboratories Inc.) with the molecular weight ranging from 420 to 22100.

2.2.5. pK_a measurement

The pK_a values of OS at different molecular weights were determined by titration method. In each case, OS were dissolved in distilled water in order to achieve 0.1 % OS solution (weight ratio) in a 200 ml beaker. The pH of solution was adjusted to 1 - 2 by NaOH 5N. After that, the pH sensitivity of OS solution was measured by recording the change of pH value when HCl 0.1 N was added into the OS solution. The change of pH value was recorded by pH meter and the titration curve was then built. According to the titration curve, the pK_a value was calculated.

3. RESULTS AND DISCUSSIONS

3.1. D,L-Serine-based oligoesters structure

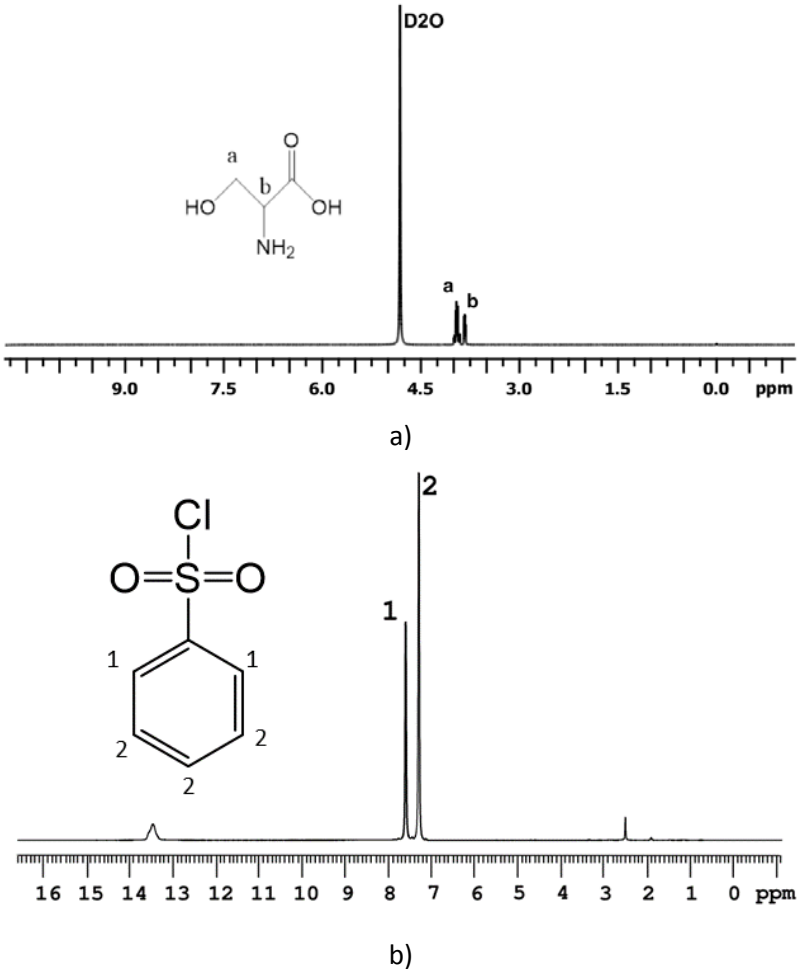


Figure 1. 1H NMR spectra of D,L-Serine a) in D2O, Benzenesulfonyl chloride b).

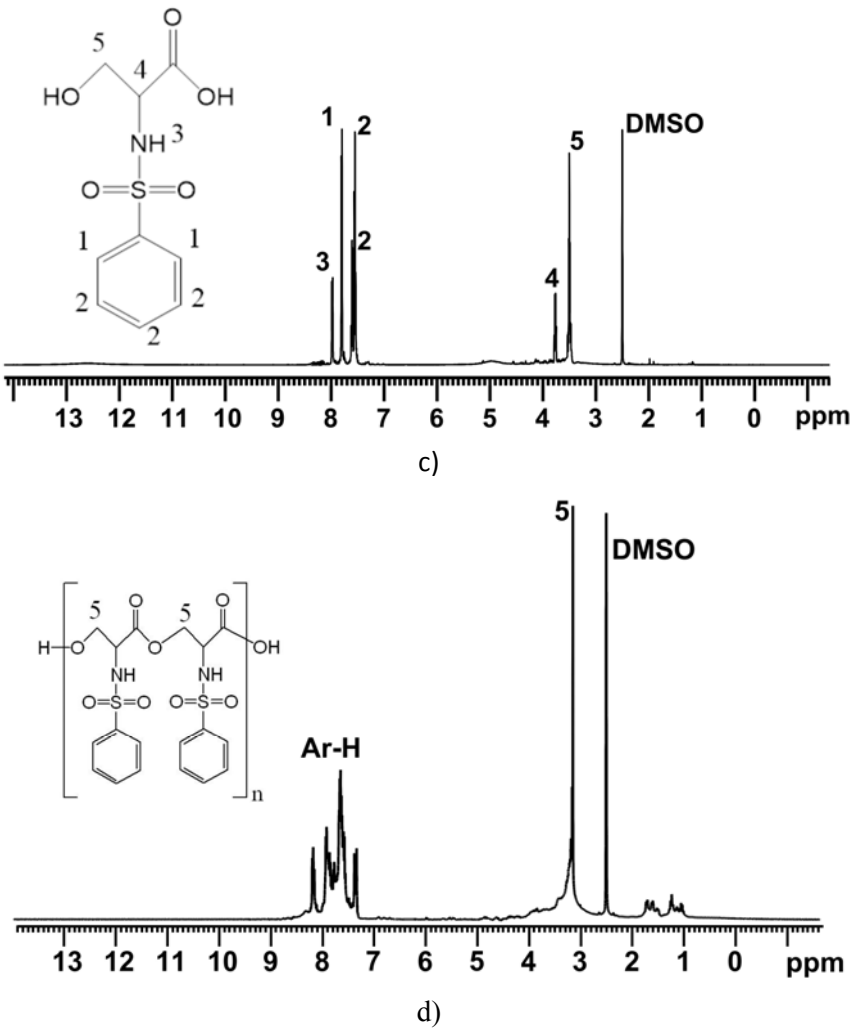


Figure 1. 1H NMR spectra of mSerine (c) and OS (d) in DMSO.

In order to confirm the successful modification, ^{1}H-NMR spectrums of pure D,L-Serine and Benzenesulfnoyl chloride were used as references (Figure 1a and b). A peak at 3.955 ppm (signal "a") represented methylene protons of D,L-Serine, and the chemical shift of methine proton appeared at 3.831 ppm (signal "b"), while aromatic protons of benzenesulfonyl chloride shifted to 7.604 ppm and 7.295 ppm (signal "1,2"). And because protons of $-NH_2$,-COOH groups are interchangeable, they did not appeared in spectrum under D_2O. Figure 1c presents the ^{1}H-NMR of mSerine after the modification, the signals representing D,L-Serine (a and b in Figure 1a, 4 and 5 in Figure 1c) and Benzenesulfonyl chloride (1 and 2 in Figure 1b and c) all appeared in the ^{1}H-NMR of mSerine, and the characteristic signal assigned to the sulfonamide protons appeared at 7.972 ppm (signal "3"). Therefore, amino groups of D,L-Serine were successfully modified with benezenesulfonyl chloride. The spectrum of OS was shown in Figure 1c. The characteristic peaks of aromatic protons (Ar-H) shifted to 7-8 ppm, while those of methylene protons appeared at 3.154 ppm (signal "5"). According to the ^{1}H-NMR spectrums, the OS was successfully synthesized by means of condensation reaction between carboxylic and

hydroxyl groups of D,L-Serine. Additionally, not many noisy signals were found in these ^{1}H-NMR spectrums, resulting in a relatively high purity of obtained oligomers.

3.2. The influence of the feed ratios of DCC/Serine (mole/mole) and DMF/Serine (v/w) on the OS molecular weight.

After synthesized, OS was purified and dried before GPC analysis in order to determine the molecular weight (Table 1)

Table 1. The molecular weight of OS (Mn) at different feed ratios.

N°	Sample	DCC/Serine (mole/mole)	DMF/mSerine (v/w)	M_n
1	9-1-2	1/2	9/1	2314
2	9-1	1	9/1	3014
3	9-2	2	9/1	5408
4	9-3	3	9/1	5722
5	14-1-2	1/2	14/1	1455
6	14-1	1	14/1	2133
7	14-2	2	14/1	2907
8	14-3	3	14/1	4438
9	20-1-2	1/2	20/1	1230
10	20-1	1	20/1	1272
11	20-2	2	20/1	1442
12	20-3	3	20/1	1790

Based on the GPC results, the influence of the feed ratios of DCC/Serine (mole/mole) and DMF/Serine (v/w) on the molecular weight exhibited in diagrams in Figure 2, molecular weight of OS rose as the mole ratio DCC/mSerine increased from 1/1 to 3/1. This trend was decided by the nature of polymerization using DCC as a coupling agent. In this case, DCC (dicyclohexylcarbodiimide) and the carboxylic acid are able to form an O-acylisourea intermediate, which offers reactivity similar to the corresponding carboxylic acid anhydride. DMAP, as a stronger nucleophile than the alcohol, then reacts with the O-acylisourea leading to a reactive amide ("active ester"). This intermediate cannot form intramolecular side products but reacts rapidly with alcohols. DMAP acts as an acyl transfer reagent in this way, and subsequent reaction with the alcohol gives the ester. Moreover, after forming an O-acylisourea intermediate with the equivalent ratio of 1/1, DCC transformed into dicyclohexylurea (DCU) as a byproduct. Hence, with the rise of DCC amount, the O-acylisourea intermediate amount went up, so the esterification accelerated. That led to the increase in OS molecular weight.

On another hand, M_n of OS also depended on the reactant concentrations. When the amount of DMF solvent went up (from 9 ml to 20 ml), it means that the reactant concentrations as well as Mn of OS fell down. This phenomena was because with the decrease reactant concentrations, the reacting possibilities of reactants went down, the esterification then took place more difficulty.

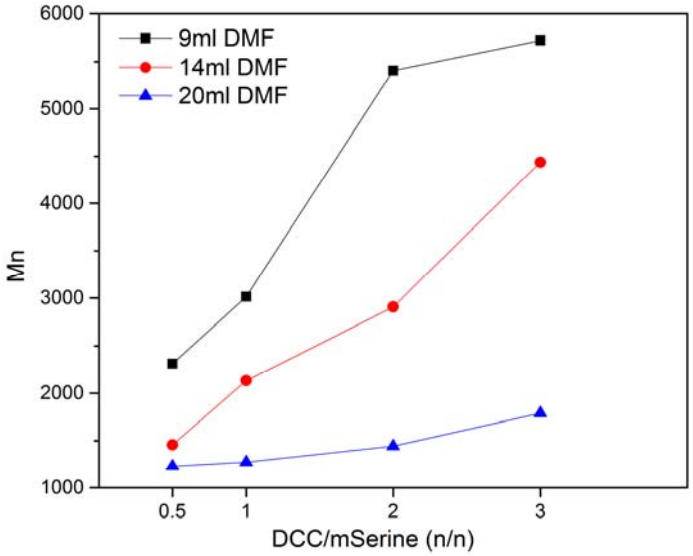


Figure 2. Diagrams of the influence of the amount of DCC and DMF on Mn of OS.

In addition, the concentrations of elements directly affected on the polydispersity index (PDI) of oliesters. To be more specific, as these concentrations went down, the reactants had enough space to conduct their chemical reaction and the competition of chain prolonging was low, therefore, oligoester PDI had a tendency to decrease (Figure 3).

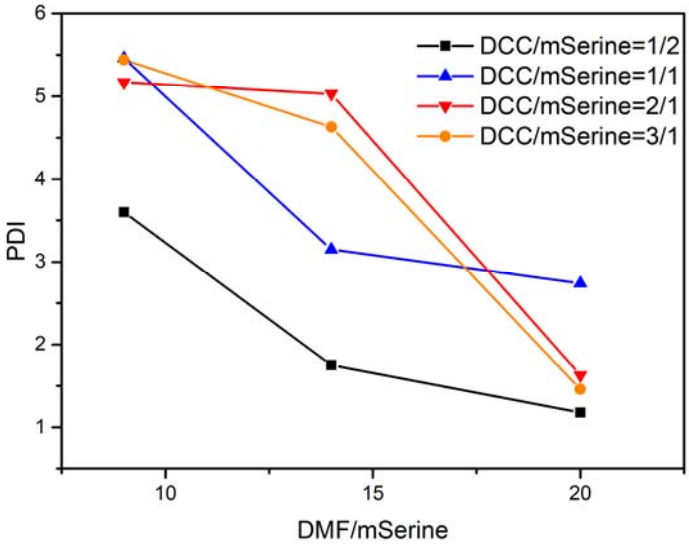


Figure 3. Oligoester PDI with the change of DMF amount.

3.3. The pH-sensitivity of oligoester

Table 2. Solubility of OS at different environmental pH.

		Solubility of OS	
		pH = 12 (NaOH 0.1N)	**pH = 2 (HCl 0.1N)**
M_n	**1230**	dissolved	undissolved
	1272	dissolved	undissolved
	1442	dissolved	undissolved
	1455	dissolved	undissolved
	1790	dissolved	undissolved
	2133	dissolved	undissolved
	2314	dissolved	undissolved
	2907	undissolved	undissolved
	3014	undissolved	undissolved
	4438	undissolved	undissolved
	5408	undissolved	undissolved
	5722	undissolved	undissolved

The solubility of OS at different environmental pH was shown in Table 2. The environmental pH was adjusted from 12-2 and pK_a values determined from titration curves varied between 6.94 and 7.29 at different molecular weights. At pH>10, OS completely dissolved, the solution was transparent. At the moment the environmental pH fell, there were tiny particles appeared in this solution. The amount of particles increased as pH decreased. And this phenomena stopped when the environmental pH reached 2 (Figure 4).

Figure 4. The change of OS solution stage with the change of pH: OS solution at pH > 10 (on the left); OS solution at pH < 4.

This phenomena represented the dissolving process of high molecular weight of OS. The ionizing process took place at pH high enough to dissolve the high Mn of oligoester. To be more

specific, sulfonamide groups within OS chains released their protons, therefore, the OS chain were negatively charged and able to dissolve in aqueous environment. At the moment pH fell, OS chains were de-ionized and became more hydrophobic and no longer dissolveed in water, they then aggregated, so tiny particles appeared.

However, at extremely high molecular weight (M_n = 2907 and above), OS absolutely lost their ability to dissolve in both acidic and basic aqeous environment. This phenomena could be explained as follows: when the OS became longer, as known as having higher molecular weight, their hydrophobicity as well as the interaction among these OS chains were stronger. This interaction was too strong to be broken by H_2O molecular through ionizing process. That is the reason why M_n OS was 2907 and above could not dissolve in water in any environmental pH conditions.

Table 3. pKa values, ranges of sensitivity of OS at different molecular weights.

Sample	Mn	Startpoints (upper points)	Endpoints (lower points)	pK_a	Range of sensitivy
9-1-2	2314	7.38	6.5	6.94	0.88
14-1-2	1455	7.87	6.45	7.16	1.42
14-1	2133	7.62	6.58	7.1	1.04
20-1-2	1230	8.51	6.07	7.29	2.44
20-1	1272	8.25	5.93	7.01	2.32
20-2	1442	7.92	6.43	7.17	1.49
20-3	1790	7.71	6.26	6.98	1.45

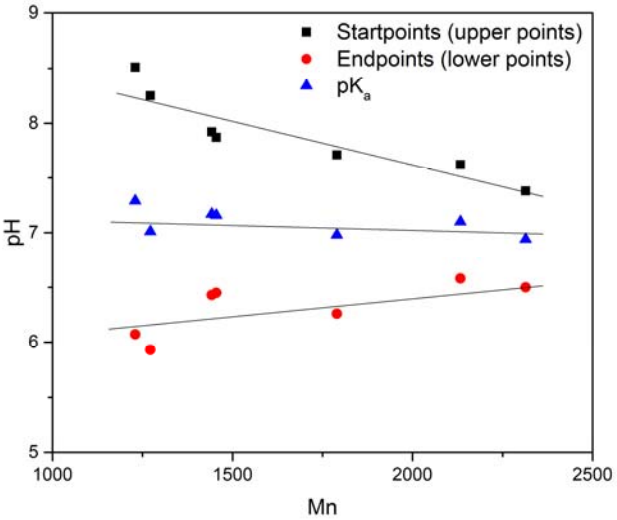


Figure 5. Startpoints (lower points) and Endpoints (upper points) and pKa values of OS at different molecular weights.

The ionizing phenomena occurred in a large range of pH, so-called “range of sensitivity” (Table 3). In addition, the fall of sensitivity range with the rise of molecular weight could be due to steric hindrance when the oilgoester chain was too large and then inhibited the ionizing process (Figure 5).

4. CONCLUSIONS

In this research, oligoester based on D,L-Serine was successfully synthesized by condensation in solution between 2 functional groups –OH and -COOH with DCC/DMAP acting as a catalyst system. The synthesized oligoester had pK_a values oscillated from 6.98-7.29. The purity of oligomers coulb be confirmed via ^{1}H-NMR, when not many noisy signals were found in these spectrums. The pH-sensitive oligoester could be combined with other stimuli sensitive polymers (as temperature, light, electric field) in order to improve drug-delivery ability and apply in injectable drug delivery systems.

Acknowledgement. This research is funded by Ho Chi Minh City University of Technology (HCMUT) under grant number TSĐH-2015-CNVL-47**.**

REFERENCES

1. Huynh D. P, Shim W. S, Kim J. H, Lee D. S. - pH/temperature sensitive poly(-ethylene glycol)-based biodegradable polyester block copolymerr hydrogels, Polymer **47** (2006) 7918-7926.
2. Osada Y., Okuzaki H., Hori H. - oltage-sensitive magnetic gels as magnetic resonance monitoring agents. Nature **355** (1992) 242 – 243.
3. Chen G., Hoffman A. S. - Graft copolymers exhibit temperature-induced phase transition over a wide range of pH. Nature **373** (1995) 49-52.
4. Huynh D.P, Ty L.V., - Synthesis of triblock copolymer of pcla-pcl-pcla base on PEG, Journal of Science and Technology **49** (2012) 112-116.
5. Qiu Y., Park K. - Environment-sensitive hydrogels for drug delivery. Adv Drug Delivery Rev. **53** (2001) 321-339.
6. Huynh D.P, Khiet H.D., - Polyamide based pH-sensitive Polymers, Journal of Science and Technology **49** (2012) 124-129.
7. Huynh D.P, Ty L.V., - Control of sol – gel phase transition diagram of temperature-sensitive PCLA-PEG-PCLA triblock copolymer hydrogel, Journal of Science and Technology **49** (2012) 117-123.
8. Shim WS, Yoo JS, Bae YH, Lee DS. - Novel injectable pH and temperature sensitive block copolymer hydrogels. Biomacromolecules **6** (2005) 2930 - 2934.
9. Kim M. S., Lee D. S., Choi E. K., Park H. J. , Kim J. S. - Modulation of poly(b-amino ester) pH-sensitive polymers by molecular weight control. Macromol Res. **13** (2005) 147-151.
10. Huynh. D. P., Huynh. C. T., Lee D. S. - Picolyamine Based pH/Temperature Sensitive Hydrogels. Macromolecular Research **18** (2010) 589-595.
11. Philip H., Puping P., - polymer-supported syntheses of some cyclic oilgoamides and some cyclic alternating oligo (amide-ester)s, polymer **40** (1999) 1871-1879.

TÓM TẮT

OLIGOESTER NHẠY PH TRÊN CƠ SỞ D,L-SERINE

Nguyễn Trí Đăng[1], Huỳnh Đại Phú[1, 2, *]

[1]*Khoa Công nghệ vật liệu, Đại học Bách khoa Tp.HCM, HCMUT–VNUHCM, 268 Lý Thường Kiệt, phường 14, quận 10, Tp.HCM*

[2]*Phòng thí nghiệm trọng điểm Polymer và Composite, HCMUT–VNUHCM, 268 Lý Thường Kiệt, phường 14, quận 10, Tp. HCM*

[*]Email: *hdphu@hcmut.edu.vn*

Trong những năm gần đây, polymer nhạy pH/nhiệt độ ngày càng thu hút được nhiều sự quan tâm nghiên cứu áp dụng trong các hệ thống vận chuyển thuốc. Trong nghiên cứu này, mục tiêu chính là tổng hợp oligomer nhạy pH. Oligomer được tổng hợp từ phản ứng trùng ngưng của các nhóm carboxylic và hydroxyl của D,L-Serine. Mặt khác, nhóm amine của amino acid này được biến tính với benezenesulfonyl chloride để tạo thành nhóm chức sulphonamide có khả năng nhạy cảm pH. Các khối lượng phân tử khác nhau của oligomer được tạo thành bằng cách điều chỉnh tỉ lệ mol giữa N,N'-Dicyclohexylcarbodiimide (DCC)/Serine (DCC là coupling agent) và tỉ lệ giữa (v/w) Dimethylformamide (DMF)/Serine (DMF là dung môi). Các oligomer được phân tích bằng ^{1}H-NMR và khối lượng phân tử của chúng được xác định bằng Gel permeation chromatography (GPC). Thêm vào đó, các giá trị pK_a của oligoesters được tính toán bằng phương pháp chuẩn độ. Tính chất nhạy pH này có thể được ứng dụng vào các hệ thống vận chuyển thuốc nhảy cảm tác nhân môi trường.

Từ khóa: nhạy pH, D,L-Serine, benezenesulfonylchloride.