

SYNTHESIS AND CHARACTERIZATION OF pH-SENSITIVE POLYMER VIA CARBOXAMIDE GROUP

**Nguyen Thi Thanh Hien^{1,2*}, Bui Phan Minh Chau¹,
Ha Cam Anh², Huynh Dai Phu²**

¹*Ho Chi Minh City University of Food Industry*

²*Ho Chi Minh City University of Technology*

*Email: hienntthanh@hufi.edu.vn

Received: 3 August 2020; Accepted: 18 March 2021

ABSTRACT

Nowadays, pH-sensitive polymers are more and more interesting in the field of novel drug delivery systems. The important features of these polymers are the change of their physical or chemical properties response to changes in environmental pH. In this study, a series of pH-sensitive polymers (OSA) were synthesized from Suberic acid and Ethylenediamine. The chemical structure of OSA was determined by ¹H NMR and FTIR. Thanking to carboxamide groups in chain of the polymers, OSA are polyanions that can release proton at high pH and accept proton at low pH. The pKa and pH sensitive range of OSA were evaluated by titration method. The result revealed the effect of DMF solvent to the pH sensitive range of OSA. Importantly, OSA-2 possessed a pH range from 7.2 to 6.3 and obtained low polydispersity index (PI), high zeta potential value. It is thus concluded that OSA-2 is promising a component of dual pH/temperature sensitive copolymers applied as an injectable drug carrier.

Keywords: pH sensitive, carboxamide, drug carrier.

1. INTRODUCTION

Intelligent polymers are a type of polymers that respond to slight changes in the environment. Characteristic responses of the polymers are showed as changes of shape, surface activity, solubility and sol to gel transition. The environmental trigger factors behind these changes can be temperature, pH, solvent, ionic strength, chemical agents, light, electrical field, and magnetic field [1, 2]. Among smart polymers, pH-responsive polymers attract much attention and have become very popular. These special properties of pH-sensitive polymers consequently make them very valuable in various applications such as drug delivery, gene delivery, sensors, membranes and chromatography.

The important feature of pH-sensitive smart polymers is able to accept or release protons in response to changes in environmental pH. This is a result of containing weak acidic or basic groups in their structure. Based on the ionizable groups, pH-responsive intelligent polymers can be classified as anionic or cationic polymers [3, 4]. In general, anionic polymers called polyanions or polyacids are ionized at pH above the pKa of the polymers network, whereas cationic polymers called polycations or polybases are ionized at pH below the pKa. According to [1-3], there is a list of examples of polyanions with characteristic acid groups such as carboxylic, sulfonic, boronic groups, in contrast of cationic polymers which hold amine, morpholino, pyrrolidine, imidazole, piperazine, and pyridine groups. Specially, the pH-sensitive polymers containing amide groups (such as

carboxamide, sulfonamide) in their structures have more interesting in pharmaceuticals because they constitute the backbone of the biologically crucial proteins [5]. Among that, the reports of application of pH-sensitive polymers having carboxamide groups indicated the size, size distribution, release drugs which controlled rigidly by the pH of the environment [6, 7].

In addition, the ionizable groups in pH-sensitive polymer structures act as hydrophilic part or hydrophobic part. Therefore, the polymers can be converted soluble-insoluble state based on the ionizing and de-ionizing ability of them. This is relative to the change of electrical charge in the polymeric molecules. For example, polyanions are ionized at high pH, the chain of polymers is expanded by electrostatic repulsion of the negatively charged groups, so the polymers are more hydrophilic. Decreasing pH value of environment, the charges decrease to neutralize that increases hydrophobic part of the polymers [2].

In this study, pH-sensitive polymers (OSA) with carboxamide groups (-CONH) were synthesized from Suberic acid and Ethylenediamine. This functional carboxamide group is considered as weak acid because the hydrogen atom of the amide group can be ionized to release a proton in solution. With the purpose of using OSA as a pH-sensitive component in order to combine with thermal sensitive polymers form dual-sensitive copolymers which are applied in injectable drug delivery system, the desired OSA can be deionized at near pH body (7.4) and ionized at above or under this pH. That makes the dual - sensitive smart copolymers effectively control the complex environmental parameters of body. In the present work, the influence of DMF solvent to obtain pH-sensitive polymers was evaluated. ¹H NMR (proton nuclear magnetic resonance) and FTIR (Fourier transform infrared spectroscopy) spectra were used to analysis chemical structure of the polymers. Characterization of physicochemical properties of OSA such as: Zeta potential, size distributions, pKa values and pH-sensitive range was determined.

2. MATERIALS AND METHODS

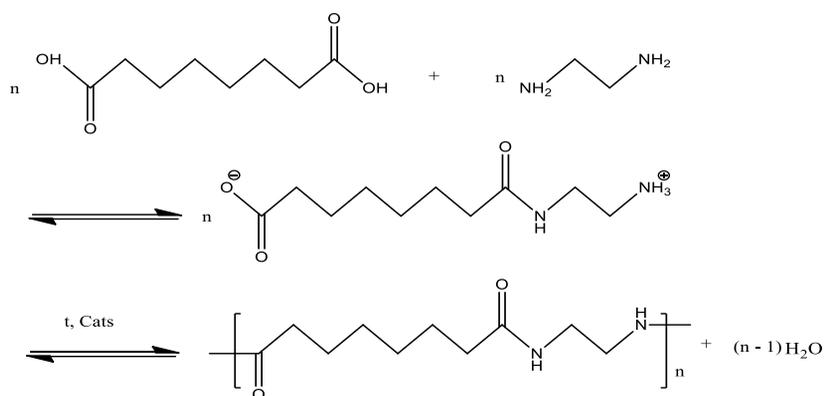
2.1. Materials

Suberic acid, Ethylenediamine were bought from Sigma-Aldrich as precursor materials. 4-Dimethylaminopyridine (DMAP) (99%) and 1,3-Dicyclohexylcarbodiimide (DCC) (99%) were purchased from Aladdin (China), they were used as catalysts system of polymer condensation reaction. Dimethylformamide (DMF) solvents were supplied from Prolabo.

2.2. Methods

2.2.1. Synthesis of OSA polymers

At beginning, the ratio of feed designated on the plan of Suberic acid/Ethylenediamine/DCC/DMAP was 1/1/0.1 (mole/mole) with DCC and DMAP as catalyst. DMF was used as solvent with various ratios. Next, the process was applied two steps: (1) Mixing Suberic with DCC, DMAP in DMF solvent to become completely solution, (2) injecting dropwise of Ethylenediamine liquid into the aqueous solution at the first stage. The reaction was occurred in Nitrogen gas environment at 90 °C temperature in 4 h. Finally, the mixture was purified by using excess diethyl ether and dried under vacuum at 50 °C for 12 h.



Scheme 1. Synthesis of OSA polymers

2.2.2. ^1H NMR analysis

The ^1H -NMR spectra were obtained from Bruker Avance machine at 500MHz and used to determine the molecular structures of the polymers. Spectra of OSA were analyzed in DMSO solvent containing 0.03 % (v/v) Tetramethylsilane (TMS).

2.2.3. FTIR analysis

The IR data were recorded on a Shimadzu FT IR-8400 S spectrophotometer. The polymers were mixed with KBr and pressed to a plate for measurement.

2.2.4. Zeta potential and size distributions measurement

Dynamic light scattering (DLS) method was conducted at 25 °C with 90° scattering angle on HORIBA SZ-100 to determine the Zeta potential and size distributions of the polymers.

2.2.5. pKa measurement

The pKa values of OSA were determined by titration method. Firstly, OSA was dissolved in PBS buffer in order to attain 0.1% (w/w) concentration. Next, using NaOH 5N adjusted the pH of the solution to 10-12. After that, pH data was collected by a pH meter when adding slowly HCl 0.1 N into the OSA solution. The titration curve was built by the change of the pH value. Finally, the pKa value of OSA is determined by plotting pH titration curve [8, 9].

3. RESULTS AND DISCUSSION

3.1. Synthesis of OSA polymers

The chemical structure of OSA was characterized by ^1H NMR spectrum (Fig. 1c). The ^1H NMR spectra of Suberic acid and Ethylenediamine were used as references (Fig. 1a,b) [10]. Obviously, data of OSA showed the resonance signals at 2.16 ppm (peak 1, $-\text{CH}_2\text{CO}$), 1.49 ppm (peak 2, $-\text{CH}_2\text{CH}_2\text{CO}$) and 1.23 ppm (peak 3, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}$) which are attributed to the protons of Suberic acid moieties. Addition, the signal at 2.9 ppm (peak 5) belongs to photons of Ethylenediamine ($-\text{CH}_2\text{NHCO}$). Specially, the signal at 8.00 ppm

(peak 4) appears in the spectrum which is assigned to the protons of the amide (-NHCO) by hydroxyl unit of Suberic acid linking to amine unit of Ethylenediamine.

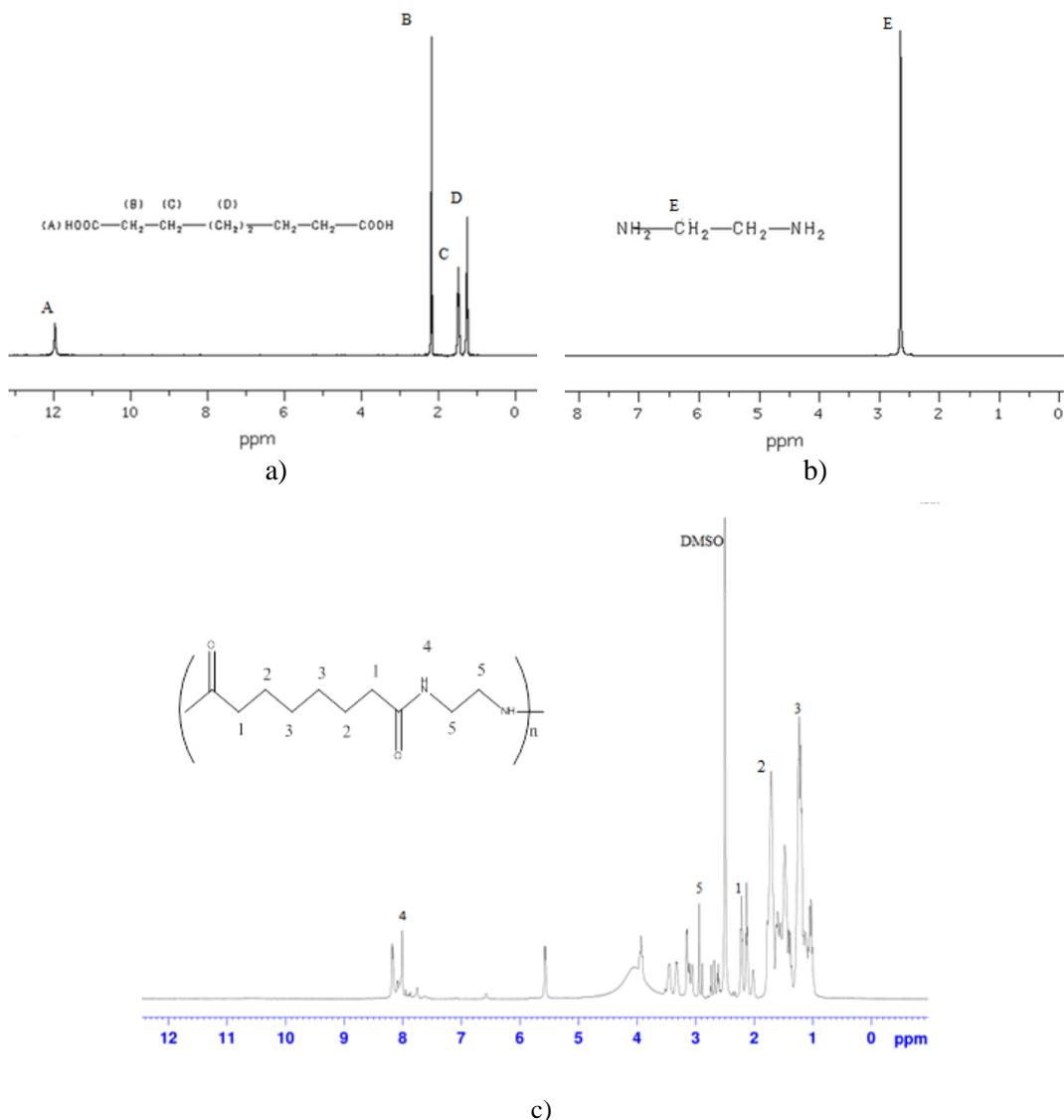


Figure 1. ¹H NMR spectrum of a) Suberic acid, b) Ethylenediamine and c) OSA in DMSO

Besides, FTIR spectrum of OSA in Fig.2 shows typical absorption peaks of obtained amide (-CONH) group. Specifically, broad band in the region of 1650 cm^{-1} - 1553 cm^{-1} correspond to the carbonyl group (-C=O) and the amine group (-N-H) of amide. Clearly, compare to the FTIR data of Suberic acid and Ethylenediamine, the spectrum of OSA displays significant changes. A characteristic absorption peak of hydroxyl (-O-H) at 3600 cm^{-1} and double peaks of amine (-N-H) at 3400 cm^{-1} present in spectra of Suberic acid and Ethylenediamine respectively, whereas in the spectrum of OSA, a broad absorption in the region $3500\text{-}3200\text{ cm}^{-1}$ results from overlap between hydroxyl (-O-H) of Suberic acid and amine (-N-H) of Ethylenediamine. Addition, a strong band at 1750 cm^{-1} of the carbonyl group (-C=O) of Suberic acid is substituted by a band at 1650 cm^{-1} in the data of OSA. Other absorption peaks around 2900 cm^{-1} , 1250 cm^{-1} of C-H bond and C-O bond in OSA spectrum respectively are similar to those of native Suberic and Ethylenediamine [10].

These results demonstrate that the OSA polymers were synthesized successfully.

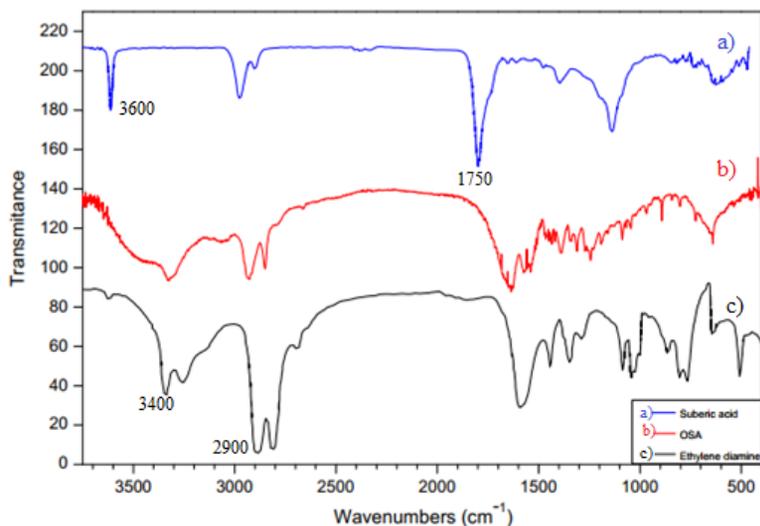


Figure 2. FTIR spectra of Suberic acid, OSA and Ethylenediamine

3.2. The influence of DMF solvent to a range of pH sensitive and pKa of OSA

Carboxamide formation from condensation reaction is often more convenient achievement through application of a suitable coupling agent. DMF has often selected as solvent for kind of this reaction because it displays a good reaction media [11]. Therefore, DMF was also used as solvent for synthesis OSA. Note that, if solvent is used too small in a reaction, it does not dissolve completely solution. On the contrary, consuming large amount of solvent causes decreasing concentration of reactants so reaction occurs more difficulty. This explanation shows that DMF solvent can influence to features of the formed OSA. In this study, the range of pH sensitive and pKa of OSA were evaluated following the volume of DMF solvent. Five kinds of OSA denoted in order of OSA-1, OSA-2, OSA-3, OSA-4 and OSA-5 were synthesized with ratios of DMF/reactants (v/w) corresponding 3, 5, 8, 10 and 25.

As shown in Fig.3, all of the OSA dissolved absolutely at high pH ($\text{pH} > 10$) to form the transparent solution. Decreasing pH value, the precipitation phenomena appeared which some tiny particles created in the solution. So, it can confirm that OSA are polyanions. That mean, the carboxamide groups lose protons at basic pH values, the electrostatic repulsion of the negatively charged groups makes the hydrophilic part of OSA controlling the process. The reason is the oxygen atoms of carbonyl group ($-\text{C}=\text{O}$) which have high electron negativity capture electrons from the carbon atom. The electron deficiency of the carbon atom causes it draws the electrons of $-\text{NH}$ bond, resulting in ionization. In the contrary, at the low pH, the ionizable groups accept protons resulting in the uncharged macromolecule, hydrophobic interactions of OSA dominate [2, 9].



Figure 3. The images of OSA with: pH > 10 (on the left) and pH < 4 (on the right)

From observation, OSA-1 was formed difficultly by low solvent, high viscosity, so it was not mentioned for next testing. The results of pH sensitive distance of OSA are displayed in Table 1. Exception OSA-2 has a narrow range of pH sensitive, the others have a larger pH range (>1.0 unit pH). When DMF content increased, the esterification occurred harshly by low reactant concentration. That caused the number of formed functional groups in the chain of OSA was small. So the pH of the environment changes more quickly within the pH sensitivity range of the polymer. As a result, the pH distance is wider. This is appropriate to the pKa values of the polymers that the pKa of OSA-3,4,5 is higher than of OSA-2. In Fig. 4, the pKa value of OSA-2 shows at 6.7 in the titration curve, while those of OSA-3,4,5 are 6.9, 6.9 and 7.2 respectively. Moreover, used volume of HCl to obtain the equivalence point of OSA-2 is more than of the others. All of these demonstrate the ionizable groups in the chain of these polymers decrease with increasing DMF volume.

Table 1. Ranges of pH sensitivity of OSA

Samples	$V_{DMF}/m_{reactant}$	Startpoints (upper points)	Endpoints (lower points)	Range of sensitivity
OSA-2	5	7.2	6.3	0.9
OSA-3	8	7.50	6.3	1.2
OSA-4	10	7.5	6.4	1.1
OSA-5	25	7.8	6.5	1.3

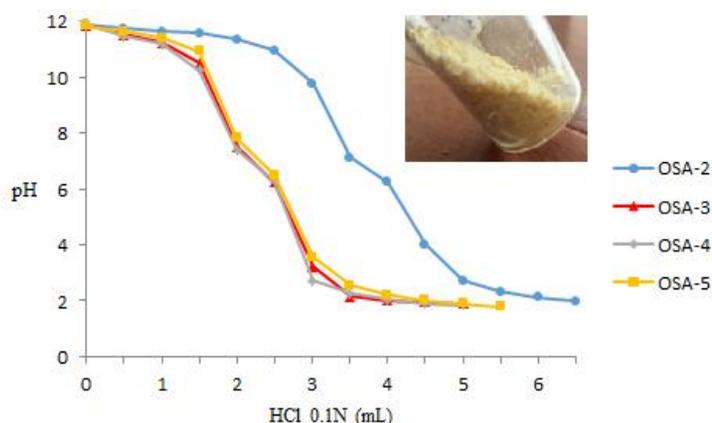


Figure 4. Diagram of pKa value change at different DMF volume

3.3. Size distribution of pH-sensitive OSA

Based on the results of size distribution of OSA showed in Fig.5, OSA-2 presents a good PI (polydispersity index) (< 0.5), whereas PI values of OSA-3,4,5 are higher than 1.5. PI values of the polymers have a tendency to increase when DMF volume increases. It is suggested that is a result of the poor product quality by tough reaction in large amount of DMF.

In addition, the zeta potential of OSA-2 in DMSO solvent at 25 °C obtained -35.3 mV in Fig.6. Obviously, OSA-2 exhibited high negative charge by the release of protons of carboxamide groups in its structure. With high surface charge and low PI, the pH-sensitive OSA-2 also displays high dispersion stability by preventing aggregations and homogeneous size [12].

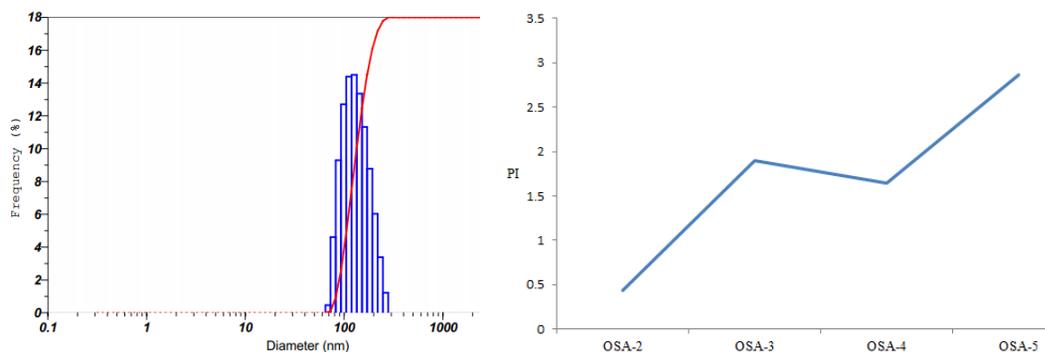


Figure 5. The PI of OSA-2 on the left and diagram of PI of different OSA on the right

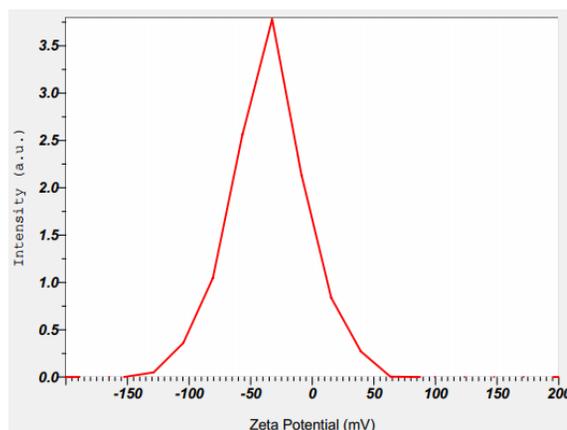


Figure 6. Zeta potential of OSA-2

4. CONCLUSION

pH-sensitive polymers (OSA) were successfully synthesized by condensation reaction. The ^1H NMR and FTIR data demonstrate the formation of carboxamide groups in the OSA structure. They are polyanions which dissolve perfectly into solution at high pH and precipitate at low pH. Ratio of DMF solvent in the reaction affected to pKa of OSA. The increasing DMF volume caused increasing pKa values. OSA-2 has a pKa value at 6.7 with the pH-sensitive start point at 7.2 (near pH body) and uniform size distribution and good dispersion stability. Thereby, OSA-2 is a potential pH-sensitive polymer that can combine with temperature-sensitive polymers to form dual pH/temperature responsive copolymers with the purpose for injectable drug delivery system.

REFERENCES

1. Kocak G., Tuncer C., Bütün V. - pH-Responsive polymers, *Polymer Chemistry* **8** (1) (2017) 144-176.
2. A. Mahajan and G. Aggarwal. - Smart polymers: innovations in novel drug delivery, *International Journal of Drug Development & Research* **3** (3) (2011) 16-30.
3. Almeida H., Amaral M. H., and Lobão P. - Temperature and pH stimuli-responsive polymers and their applications in controlled and self-regulated drug delivery, *Journal of Applied Pharmaceutical Science* **2** (6) (2012) 01-10.
4. Xie J., Li A., and Li J.. - Advances in pH-Sensitive polymers for smart insulin delivery, *Macromolecular Rapid Communications* **38** (23) (2017) 1–14.
5. Lundberg H., Tinnis F., Selander N., and Adolfsson H. - Catalytic amide formation from non-activated carboxylic acids and amines, *Chemical Society Reviews* **43** (8) (2014), 2714-2742.
6. Cao J., Su T., Zhang L., Liu R., Wang G., He B., and Gu Z. - Polymeric micelles with citraconic amide as pH-sensitive bond in backbone for anticancer drug delivery, *International Journal of Pharmaceutics* **471** (1-2) (2014) 28–36.
7. G. Liu, M. Li, C. Zhu, Q. Jin, Z. Zhang, J. Ji. - Charge-conversional and pH-sensitive PEGylated polymeric micelles as efficient nanocarriers for drug delivery, *Macromolecular Bioscience* **14** (9) (2014) 1280-1290.
8. Tri D. N. and Huynh D. P. - D,L-serine based pH-sensitive oligoester, *Journal of Science and Technology* **54** (5A) (2016) 45–55.
9. Ho D. K., Nguyen D. T., Thambi T., Lee D. S., and Huynh D. P. - Polyamide-based pH and temperature-responsive hydrogels: Synthesis and physicochemical characterization, *Journal of Polymer Research* **26** (1) (2019).
10. Spectral Database for Organic Compounds, (SDBS)
https://sdfs.db.aist.go.jp/sdfs/cgi-bin/cre_index.cgi.
11. MacMillan D., Murray J. , Sneddon H., Jamieson C., Watson A. - Evaluation of alternative solvents in common amide coupling reactions: Replacement of dichloromethane and N,N-dimethylformamide, *Green Chemistry* **15** (3) (2013).
12. Hanaor D., Michelazzi M., Leonelli C., Sorrell C. - The effects of carboxylic acids on the aqueous dispersion and electrophoretic deposition of ZrO₂, *Journal of the European Ceramic Society* **32** (1) (2012) 235–244.

TÓM TẮT

TỔNG HỢP VÀ CÁC ĐẶC TÍNH CỦA POLYMER NHẠY pH BỞI NHÓM CARBOXAMIDE

Nguyễn Thị Thanh Hiền^{1,2*}, Bùi Phan Minh Châu¹,
Hà Cẩm Anh², Huỳnh Đại Phú²

¹Trường Đại học Công nghiệp Thực phẩm TP.HCM

²Trường Đại học Bách khoa TP.HCM

*Email: hienntthanh@hufi.edu.vn

Ngày nay, polyme nhạy pH rất được quan tâm trong lĩnh vực truyền dẫn thuốc. Các tính năng quan trọng của các polyme này là tính chất vật lý hoặc hóa học của chúng có thể thay đổi theo pH môi trường. Trong bài báo này, một loạt các polymer nhạy pH (OSA) được tổng hợp từ axit suberic và ethylenediamine. Cấu trúc hóa học của các OSA được xác định bằng ¹H NMR và FTIR. Với sự hình thành nhóm chức carboxamide trên mạch nên OSA là polyanion nhạy pH, do đó nó có thể nhả proton ở pH cao và nhận proton ở pH thấp. Khoảng nhạy pH và pKa của các OSA được xác định bằng phương pháp chuẩn độ. Kết quả thu được cho thấy sự ảnh hưởng của dung môi DMF lên pKa và khoảng nhạy pH của OSA. Đặc biệt, OSA-2 có khoảng nhạy pH từ 7,2 đến 6,3, kích thước đồng đều và ổn định phân tán tốt. Do đó, có thể nói OSA-2 có triển vọng là thành phần của polymer nhạy pH/nhiệt độ được ứng dụng như chất dẫn truyền thuốc dạng tiêm.

Từ khóa: Nhạy pH, carboxamide, dẫn truyền thuốc.