SYNTHESIS AND CHARACTERIZATION OF POLY(VINYL PHOTPHONIC ACID)-CHITOSAN USING TETRAETHYLENE GLYCOLDIMETHACRYLATE CROSSLINKER

TỔNG HỢP VÀ ĐẶC TÍNH CỦA HYDROGEL POLY (VINYL PHOTPHINIC AXIT)-CHITOSAN TRONG SỰ CÓ MẶT CỦA TETRAETHYLENE GLYCOLDIMETHACRYLATE

Trinh Duc Cong[1,*], Nguyen Thi Thuc[1], Do Truong Thien[1], Tran Thi Y Nhi[1], Pham Thi Bich Hanh[1], Le Quang Tuan[1], Le Thi Thanh Ha[1], Lai Thi Thuy[1], Nguyen The Huu[2]

ABSTRACT

In this study, poly(vinylphosphonic acid)-chitosan (PVPACS) hydrogel was prepared via in situ polymerization of vinyl phosphonic acid (VPA) and chitosan (CS) in the presence of tetraethylene glycoldimethacrylate (TEGMA) crosslinking agent agent with amoni pesunphat APS as initiators. Characteristics of hydrogel were determined by the swelling, gelation time, fourier transform infrared spectrum (FTIR), thermogravimetric analysis (TGA) and calcium chelation test. The results showed that the swelling and gelation time behavior increased with the increase of VPA contents. FTIR spectroscopy clearly indicated the proton exchange reactions between VPA and CS forming ionic crosslinks. PVPACS has an ability to chelate form between acid groups with Ca^{2+} ions from the surrounding environment ion. At 30% VPA contents, the maximum adsorption capacity of Ca^{2+} was 90.5mg/g at pH = 7 and 125.7mg/g at pH = 10, respectively. Calcium chelation test showed potential for bone tissue engineering applications.

Key words: *Poly(vinyl photphonic axit)-chitosan, hydrogel, vinyl photphonic axit, Chitosan.*

TÓM TẮT

Trong nghiên cứu này, hydrogel PVPACS đã được tổng hợp từ vinyl photphonic axit (VPA) và chitosan(CS) trong sự có mặt của chất tạo lưới tetraethylene glycoldimethacrylate (TEGMA) với chất khơi mào amoni pesunphat (APS). Đặc trưng tính chất của PVPACS đã được xác định thông qua độ trương, thời gian gel hóa, phổ hồng ngoại FTIR, phân tích nhiệt trọng lượng TGA và khả năng liên kết với ion Ca^{2+}. Kết quả nghiên cứu cho thấy, độ trương và thời gian gel hóa của hydrogel tăng khi tăng hàm lượng VPA. Trong phổ FTIR của PVPACS đã có sự hình thành liên kết cộng hóa trị và liên kết ion giữa các nhóm chức của CS và VPA. Các nhóm chức của PVPACS có khả năng liên kết phức với ion Ca^{2+} trong dung dịch và với hàm lượng VPA là 30% thì khả năng liên kết lớn nhất với ion Ca^{2+} là 90,5mg/g tại môi trường trung tính có pH = 7 và 125,7mg/g tại môi trường kiềm có pH = 10. Thông qua khả năng liên kết với ion Ca^{2+} đã bước đầu định hướng ứng dụng của PVPACS trong kỹ thuật y sinh.

Từ khoá: *Poly(vinyl photphonic axit)-chitosan, hydrogel, vinyl photphonic axit, Chitosan.*

[1]Institute of Chemistry, Vietnam Academy of Science and Technology
[2]Faculty of Chemical Technology, Hanoi University of Industry
[*]Email: congvhh@gmail.com
Received: 10/9/2021
Revised: 14/10/2021
Accepted: 15/11/2021

1. INTRODUCTION

Chitosan $(C_6H_{11}O_4N)_n$ is a natural polymer with nontoxicity and biodegradation properties [1]. Chitosan is also known as an agent that stimulates the growth of osteoblasts around the implant site, thus making bone wounds regenerate faster [2]. Many researches were based on chitosan hydrogels have been shown many prospects not only for burns but also for wound healing, cartilage regeneration, artificial kidney, drug delivery,... [1-4]. In chemical structure, chitosan has positively charged amine groups, so it can interact with negatively charged groups, which forms its regenerative properties [5].

Poly(vinylphosphonic acid) (PVPA) is synthesized by free radical polymerization of vinylphosphonic acid (VPA) monomer or saponification of VPA methyl ester monomers followed by hydrolysis [6]. The phosphonic acid groups of PVPA has been shown to be beneficial due to its similarity to the phosphonic acid groups of natural bone hydroxyapatite. On the other hand, the structure of PVPA is similar to bisphosphonates, a class of drugs used in the treatment of osteoporosis [4, 7]. PVPA hydrogels bind avidly to divalent calcium ions on the bone mineral surface, potentially enhancing bone mineralisation [8].

The objective of this study was to synthesize poly(vinyl phosphonic acid) - chitosan hydrogels (PVPACS) based on vinyl phosphonic acid and chitosan in the presence of tetraethylene glycol dimethacrylate (TEGMA) agent. In particular, the calcium chelation of PVPACS hydrogels has been studied extensively for use in biomedical applications.

2. EXPERIMENTAL

2.1. Materials

Chitosan (CS) was purchased from Sigma Aldrich, deacetylated ≥ 75%, Vinylphosphonic acid (VPA) was purchased from Sigma Aldrich, purity > 97%, tetraethylene glycoldimethacrylate (TEGMA) was Sigma Aldrich reagent grade, purity > 97%, amoni pesunphat (APS) purity > 99% (Sigma Aldrich), acetic acid purity > 99% (Sigma Aldrich), NaOH purity, HCl purity.

2.2. Preparation of poly(vinylphosphonic acid) - chitosan (PVPACS) hydrogel

The following method details the synthesis of PVPACS. Further details of the experimental conditions and procedures for all compositions are presented in Figure 1.

First, chitosan 2% solution was prepared by dissolving 10g of purity chitosan in 500ml of acetic acid 1% solution.

Then, PVPACS was prepared. VPA solution with different concentrations (10ml) was dissolved completely followed by slowly adding 5ml of CS 2% solution in the presence of APS 1% (w/w) solution as initiator and TEGMA 2% (w/w) as crosslinking agent. The polymerization process of VPA and CS was stirred at 75^{0}C temperature, 180 minutes time.The resultant polymer was washed with distilled water and then dried in a vacuum at 70^{0}C.

Gelation time was determined from the blend components were mixed until the reaction liquid stopped moving [6].

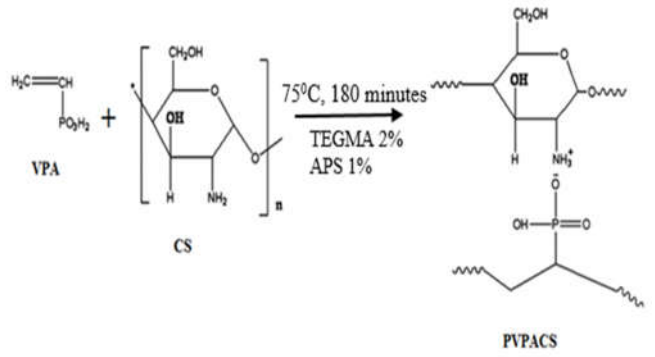

Figure 1. The polymerization process of VPA with CS to produce PVPACS

2.3. Characterization of PVPACS hydrogels

- *Fourier transform infrared spectroscopy (FTIR):* FTIR data of polymers were collected from 4000 to 500 cm^{-1} using IMPACT 400 - Nicolet (USA) instrument at Institute of Chemistry- Vietnam Academy of Science and Technology

- *Thermal Gravimetric Analyzer TGA*: TGA of the polymers were examined by 60 Shimadzu at Institute of Material- Vietnam Academy of Science and Technology. The samples were heated from room temperature to 700^{0}C under N_2 atmosphere at a scanning rate of 10^{0}C/min.

- *Swelling degree of hydrogel:* The mass increase was measured by weighing the hydrogel removed from the swelling media at certain times. Swelling characterization was also done as take m (g) dry PVPACS doused in Na_2HPO_4 0.1M solution and adjusting the pH 7.0 and 10.0 of the solutions with NaOH 0.1M. Hydrogels were kept 24 h to determine the effect of medium pH on the swelling behavior. The swelling studies of hydrogel were performed in triplicate. The % swelling degree, SW (%) was calculated by [5]:

$$SW\ (\%) = (W_s - W_d)/W_d \times 100 \quad (1)$$

Where: W_s is the mass of swollen sample and W_d is the initial mass.

- *Calcium chelation capacity of PVPACS hydrogels:* The chelation capacities were obtained from the exchange of the dry PVPACS (0.2g) with 20ml of $CaCl_2$ 0.1M solution for 60 minutes on magnetic stirrer at 30^{0}C with constant speed. The chelation capacities were determined from the decrease in Ca^{2+} ion concentration in solution after equilibration. The concentration of Ca^{2+} ion was determined by ICP. Chelation capacity was calculated by the following equation (1):

$$q_e = \frac{(C_0 - C_t).V}{m} \quad (2)$$

Where: q_e is the chelation capacity (mg/g) at equilibrium; C_0 and C_t are the initial and equilibrium concentration of Ca^{2+} ion (mg/l), respectively; V is the volume (l) of solution and m is the mass (g) of adsorbent used.

3. RESULT AND DISCUSSION

3.1. Physical and chemical characterization of PVPACS hydrogels

3.1.1. Swelling hydrogel and gelation time

The swelling degree and gelation time of PVPACS hydrogel at neutral medium with pH 7.0 and alkaline medium with pH 10.0 were shown in Table 1.

Table 1. Swelling degree and gelation times of PVPACS hydrogels

VPA contents	SW (%) at pH = 7,0	SW (%) tại pH = 10,0	Gelation time (minutes)
VPA 20%	31.11	47.32	66
VPA 30%	42.53	55.40	75
VPA 40%	50.67	61.73	97
VPA 50%	55.91	70.94	102
VPA 60%	61.36	77.52	154

The swelling behavior of hydrogels depends strongly on external stimuli such as pH, ionic strength and temperature. Table 1 showed that swelling degree and gelation time increased with the increase of VPA content in the reaction [5]. Besides, pH of the medium also had an effect on the hydrogel swelling. The hydrogel swelling reaches its maximum value at pH 7 and 10. These results showed that - PO_3H group is acid and can not dissociate at low pH. At alkaline medium with pH 10.0, the acidic groups are negatively-charged and so electrostatic repulsions occur within the polymer network, which increases the swelling.

This allows increased cell infiltration as well as diffusion and has been studied extensively for using in biomedical applications.

3.1.2. FTIR spectrum

PVPACS is formed by crosslinking between the -OH groups of CS and the -P-OH groups of VPA. Further investigation into the structure of PVPACS was achieved by evaluating the FTIR spectrums presented in Figure 2.

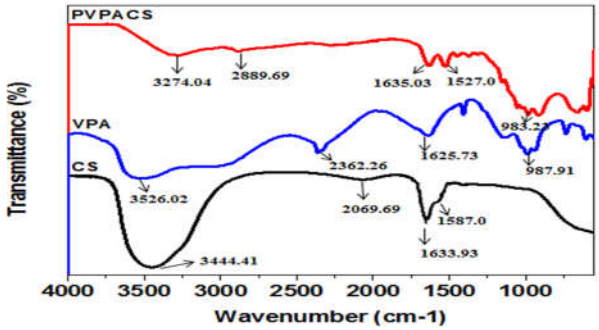

Figure 2. FTIR spectra of CS, PVA and PVPACS

Fig. 2 shows the FTIR of CS, VPA and PVPACS. In the FTIR of CS, the strong absorption band at 1633.93cm^{-1} and 1587.0cm^{-1} corresponds to the stretching vibration of –(C-O) and -NH_2 groups, respectively. The N-H and O-H stretching vibrations are also presented at 3444.4cm^{-1} [6]. The FTIR spectrum of VPA shows that the absorption peak at 1625.73 - 987.91cm^{-1} was assigned to –P=O group in phosphonic acid. The absorption peak at 3526.02cm^{-1} was attributed to the stretching vibrations of the hydroxyl group in phosphonic acid [9]. The FTIR spectrum of PVPACS hydrogel confirmed that VPA and CS crosslinking by both covalent and ionic interaction. The significant shift in absorption band 1635.03 - 1527cm^{-1} were attributed to NH^{3+} bending vibration, which shows the electrostatic interaction between VPA and CS. The covalent bonding was indicated by the presence of -CH_2-O-P-O-CH_2- stretching mode at peaks from 1000cm^{-1} to 1100cm^{-1}. Additionally, the strong absorption band at 930 - 990cm^{-1} corresponds to deprotonated phosphonic acid. This shows that there is a complexing between groups in the hydrogel through proton exchange reactions, i.e. -NH O - P- [1, 5-6]. The FTIR signals of PVPACS in Figure 2 are completely consistent with some researches [1, 5-7]. Therefore, the FTIR results provide strong evidence for the successful synthesis of PVPACS.

3.1.3. Thermal analysis

Thermogravimetric analysis curves of PVPACS from room temperature up to 700^{0}C were presented in Fig. 3.

It was previously reported that pure CS is thermally stable up to 180^{0}C. The degradation temperature of the pristine PVPA was reported to be near 200^{0}C [5]. Fig. 3 shows that the thermal degradation of VPA as following steps, firstly, the VPA exhibited a small mass loss from room temperature to 180^{0}C, implying a loss of moisture and condensation of phosphonic acid units in the branch. The major mass loss of VPA at the range 180^{0}C - 450^{0}C (48.39%). That can be decomposed of phosphonic acid units. The last weight loss above 450^{0}C was caused by the decomposition of the PVPACS network.

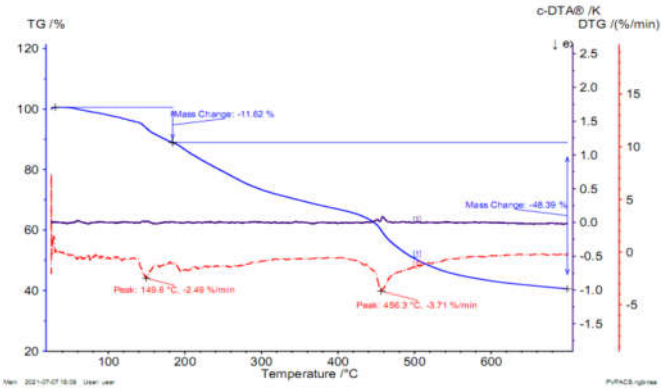

Figure 3. Thermogravimetric analysis (TGA) of PVPACS

3.2. The calcium chelation capacity of PVPACS hydrogel

The degree of dissociation of a polymer is important in terms of its ability to chelate metal ions from the surrounding environment. PVPACS contains acid groups to chelate one Ca^{2+} ions. Therefore, there exists a combination of electrostatic effects as well as a chemical association of the calcium with the negatively charged groups. The calcium chelation capacity of PVPACS at pH 7.0 and 10.0 was presented in Figure 4.

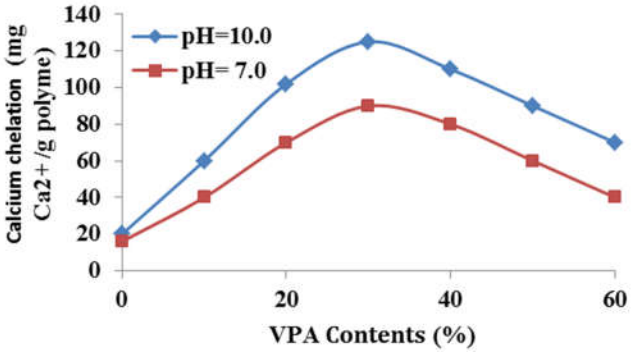

Figure 4. The calcium chelation capacity of PVPACS hydrogels at pH 7.0 and 10.0

Fig. 4 showed that calcium chelation capacity increased with the increase of VPA contents from 10 to 30%, reached the maximum at 30% VPA and then decreased steadily with the increase of VPA content up to 60%. This can be explained by the formation of both covalent and ionic bond between calcium chelation and the acid groups of PVPACS. Thus, the contents of phosphonic acid groups may also play a significant role in the polymer's ability to chelate calcium ions. Besides, there is an increase in calcium chelation with the increase of pH of the medium. As pH increases, the acid groups become increasingly deprotonated. This results in intramolecular repulsion and hence an expansion of the PVPACS chain which leads to more available binding sites for Ca^{2+} [1]. This characterization has been applied in cell cultures in order

to enhance osteogenesis of all osteoblasts. Therefore, this study has initially demonstrated that PVPACS can be used for bone tissue engineering applications [5, 7].

4. CONCLUSIONS

The aim of this work is to prepare poly(vinylphosphonic acid)-chitosan (PVPACS) hydrogel by polymerization of vinyl phosphonic acid (VPA) and chitosan (CS) in the presence of tetraethylene glycoldimethacrylate (TEGMA) crosslinking agent. Characteristics of hydrogel were determined by the swelling degree, gelation time, fourier transform infrared spectrum (FTIR), thermogravimetric analysis (TGA) and calcium chelation test. The swelling hydrogel and gelation time of PVPACS increased when VPA contents increased. Confirmation of the structure of the copolymers was provided by evaluation of their FTIR spectra. The FTIR spectrum of PVPACS hydrogel confirmed that VPA and CS crosslinking by both covalent and ionic interaction. The fabricated PVPACS has an ability to chelate between acid groups and Ca^{2+} ions. At 30% VPA monomer, the calcium chelation capacity exhibited a maximum with 90.5 (mg/g) at pH 7.0 and 125.7 (mg/g) at pH 10.0, respectively. Therefore, these results suggest that PVPACS has the potential to be used in bone tissue engineering applications.

ACKNOWLEDGEMENT

Authors would like to thank the Institute of Chemistry, Vietnam Academy of Science and Technology for financial support (Code VHH.2021.08).

REFERENCES

[1]. Celine Abueva, B. T. L., 2014. *Poly(vinyl phosphonic acid) immobilized on chitosan: A glycosaminoglycan-inspired matrix for bone regeneration*. Intrnational Journal of Biological Macromolecules, 64: p. 294-301.

[2]. N.C. Khoa, T.N. Quyen, N.D. Hai, 2013. *Tetronicbiocompatible scaffold for biomedical applications*. Journal of Biomaterials Science, Polymer Edition, p. 1-12.

[3]. Wang X, Ma J, Wang Y, He B., 2002. *Bone repairs in radii and tibias of rabbits with phosphorylated chitosan reinforced calcium phosphate cements*. Biomaterials 23:4167–76**.**

[4]. Johnson JW, Herschler A., 2011. *A review of the mechanical behavior of CaP and CaP/ polymer composites for applications in bone replacement and repair*. Acta Biomater 7:16–30.

[5]. Fadime Goktepe, S.U.C., Ayhan Bozkurt, 2008. *Preparation and the proton conductivity of chitosan/poly(vinyl phosphonic acid) complex polymer electrolytes*. Journal of Non-Crystalline Solids, 354: p. 3637-3642.

[6]. Le Quoc Tuan, Dang Hoang Phuc, Vo Van Toi, Thi Hiep Nguyen, 2015. *Fabrication of In Situ Cross-Linking Polyvinyl Phosphonic Acid - Chitosan Hydrogel for Wound Applications*, Springer International Publishing Switzerland 2015, p.317-320.

[7]. Dang Hoang Phuc, Nguyen Thi Hiep, Do Ngoc Phuc Chau, Nguyen Thi Thu Hoai, 2016. *Fabrication of Hyaluronan-Poly(vinylphosphonic acid)-Chitosan Hydrogel for Wound Healing Application*. International Journal of Polymer Science, p.1-9.

[8]. Oliveira JM, Rodrigues MT, Silva SS, Malafaya PB, Gomes ME, Viegas CA, et al., 2006. *Novel hydroxyapatite/chitosan bilayered scaffold for osteochondral tissueengineering applications: Scaffold design and its performance when seeded with goat bone marrow stromal cells*. Biomaterials 27: p.6123–37.

[9]. Nurettin Sahiner, Selin Sagbasa, 2013. *The preparation of poly(vinyl phosphonic acid) hydrogels as new functional materials for in situ metal nanoparticle preparation*. Colloids and Surfaces A: Physicochem. Eng. Aspects 418, 76–83.

THÔNG TIN TÁC GIẢ

Trịnh Đức Công[1], Nguyễn Thị Thức[1], Đỗ Trường Thiện[1], Trần Thị Ý Nhi[1], Phạm Thị Bích Hạnh[1], Lê Quang Tuấn[1], Lê Thị Thanh Hà[1], Lại Thị Thúy[1], Nguyễn Thế Hữu[2]

[1]Viện Hóa học, Viện Hàn lâm Khoa học và Công nghệ Việt Nam

[2]Khoa Hóa học, Trường Đại học Công nghiệp Hà Nội