Tạp chí Khoa học và Công nghệ 53 (5) (2015) 637-645
DOI: 10.15625/0866-708X/53/5/5995

PREPARATION, CHARACTERIZATION AND MORPHOLOGY OF CHITOSAN FILM CONTAINING NIFEDIPINE

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Received: 25 March 2015; Accepted for publication: 30 March 2015

ABSTRACT

This paper presents some characters and morphology of films based on chitosan (CS) and nifedipine (NIF) with various NIF content prepared by solution method. The characteristic peaks of some groups in CS and NIF in Fourier transform infrared spectroscopy (FTIR) spectra of the CS/NIF films shift slightly in comparison with that in the FTIR spectra of CS and NIF. This proved that CS and NIF were interacted with each other. NIF dispersed in the CS matrix with the relatively small size from 200 nm to 2 μm. Thermal analysis results showed that the CS/NIF films have less thermostable than the CS. The calibration equations of NIF were set up from data of UV-Vis spectra and regression coefficients obtained higher than 0.996. In vitro release study of CS/NIF films indicated drug release rate was almost higher in pH 7.4 solution than that in pH 6.8 solution for the samples containing 10, 30 and 50 wt% NIF.

Keywords: chitosan (CS), nifedipine (NIF), thermal property, solution method, drug release.

1. INTRODUCTION

Nifedipine (NIF) with scientific name is dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicacboxylat. It is a pharmaceutical/calcium antagonists of the dihydropyridine group having the effect of selective inhibition. NIF has characters as very low solubility, biodegradability by the photochemical reaction, ect. About pharmacokinetics, it is rapidly absorbed from the gastrointestinal tract and presents in the blood after a few minutes. Its half-life in plasma is about 3 hours and absolute bioavailability of about 50 %. There are 90 to 95 % of NIF binding with plasma protein, almost completely metabolized in the liver to metabolites inactive. In practical application, the NIF is used as an anti-hypertensive, anti-angina and Raynaud treatment [1]. However, the disadvantages of NIF are short half-life, low solubility and easy release in different pH solutions. Thus, using biopolymers such as poly(lactic acid), chitosan, polycaprolactone for carrying NIF promises to overcome these limits of NIF.

Chitin is a well known biopolymer which are derived from materials sourced natural such as fungus, algae or shell of the arthropod. Chitosan (CS) is obtainted by alkaline deacetylation of chitin. In recent years, CS is used as potential materials for the preparation of CS nanoparticles because of its advantages in nanoscale. It attracted the attention of many scientists owing to the

positive impact on cancer patients. It is used a lot in the field of pharmaceuticals and medicine due to its good properties such as non – toxic, antibacterial ability, high biological interoperability, bioharmony, biodegradable ability, and high absorption ability [2 - 6]. CS on drug delivery can be prepared by different methods such as coating, capsule shell, emulsion cross-linking, coacervation/precipitation, spray-drying, emulsion-droplet coalescence, reverse micellar method and solution casting, ect. [5]. There are many drugs which were delivered by CS including 5-ASA, propranolol-HCl, bovine serum albumin, ofloxacin, paclitaxel and so on [5].

Some manuscripts on the release of drug from CS films have published [7-10]. Bhardwaj *et al.* evaluated some physico-chemical properties and in vitro release of the different concentration chitosan based periodontal film of ofloxacin [7]. In vitro drug release data indicate that the films showed an initial burst release followed by sustained release of the drug. Dhanikula *et al.* fabricated chitosan films delivering paclitaxel at the tumor site in therapeutically relevant concentrations [8]. These films released only 10 % to 15 % of loaded paclitaxel by a burst effect under in vitro testing conditions, with lysozyme having no effect on the release. In a recent research, Liji Jacob *et al.* prepared NIF patches were prepared using polymers like CS, hydroxyl propyl methyl cellulose (HPMC), polyvinyl pyrolidone (PVP), and polyvinyl alcohol (PVA). In vitro release studies were conducted for NIF loaded patches in phosphate buffer (pH 6.8) solution. Patches exhibited drug release only about 20 % after 1 hours and up to 96.52 % for 8 hours [9]. Upadrashta *et al.* prepared buccal bilayered devices with a mixture of NIF and propranol as well as CS displayed promising potential for use in controlled delivery in the oral cavity [10].

It can be seen that the study on preparation and some properties of films contains two components CS and NIF has been not focused. Therefore, this paper presents some characters including FTIR spectra, thermal properties and morphology of CS/NIF films with different NIF contents prepared by solution method. Besides, the drug release of NIF from the above CS/NIF films immersed in solutions with different pH is also investigated and discussed.

2. EXPERIMENTAL

2.1. Materials

Chitosan (CS) with degree of deacetylation >77 %, viscosity 1220 cPs, 1.61×10^5 Da and Nifedipine (NIF) in powder with purity ≥ 98 % were obtained from Sigma-Aldrich, USA. Ethanol 98 %, acetic acid 99.5 % and sodium hydroxide, monobasic potassium phosphate are the commercial products of China.

2.2. Preparation of CS/NIF films

100 mg of CS was dissolved in 10 ml of 1 % acetic acid to obtain solution A. 5 ml of 98 % ethanol solution containing 10 mg of NIF is solution B. Solution B was added into solution A and the mixture was sonicated for 15 minutes to obtain a uniform solution. This solution was poured into the petri dish and solvents were evaporated naturally. Then, the CS/NIF film of CS containing 10 wt % of NIF (CS/NIF10) was dried under vacuum about 48 hours.

These films with proportion CS/NIF 100/30 and 100/50 were prepared similarly to CS/NIF10 sample and abbreviated by CS/NIF30 and CS/NIF50, respectively.

2.3. Characterization and morphology of CS/NIF films

Fourier transforms infrared spectroscopy (FTIR)

The FTIR spectra of CS, NIF and CS/NIF films are recorded on a Nicolet/Nexus 670 spectrometer (USA) at Institute for Tropical Technology, VAST at room temperature by 16 scans with 4 cm^{-1} resolution and wave number ranging from 400 to 4000 cm^{-1}.

Field emission scanning electron microscopy (FESEM)

FE-SEM of CS, NIF and CS/NIF films coated by platinum was conducted using a S-4800 FESEM instrument (Hitachi, Japan) at Institute of Science Materials, VAST.

Thermal gravimetric analysis (TGA)

Thermal property study was performed on a DTG 60H of Shimazu Co. (Japan) at Hanoi National University of Education under argon atmosphere from room temperature to 600 ^{0}C at a heating rate of 10 ^{o}C/min.

2.4. In-vitro drug release studies

The in-vitro NIF release tests were carried out on CS containing 10, 30 and 50 wt% of NIF. Fifty milligrams of each sample was immersed in 100 ml of phosphate buffer solution (PBS) at various pH (6.8 and 7.4) at 37 ^{o}C and placed in a incubated shaker at 120 rpm. At predetermined time intervals, 3 ml of aliquots was withdrawn and the concentration of NIF released was monitored by UV spectrophotometer (CINTRA 40, GBC, USA) at 230 nm.

The dissolution medium was replaced with fresh buffer solution to maintain the total volume. The NIF release percent can be determined by the following equation:

$$\text{Drug release [\%]} = C_{(t)}/C_{(0)} \times 100$$

where C(0) and C(t) represents the amount of NIF loaded and amount of drug released at a time t, respectively. All studies were done in triplicate.

3. RESULTS AND DISCUSSION

3.1. FTIR spectra

Figure 1 shows the FTIR spectra of the CS, NIF and CS/NIF10 film. It can be observed the appearance of characteristic peaks corresponding to stretching and bending vibrations of NH, CH C=O, C-O groups in the FTIR spectrum of CS such as N-H group stretching at 3366.76 cm^{-1}, C-H stretching and bending at 2900, 2850 and 1465 cm^{-1}, respectively, and asymmetric and symmetric stretching of C-O-C group at 1081.99 and 1259.33 cm^{-1}. Moreover, the peak of C=O stretching vibration in amide group is found at 1639.26 cm^{-1} [11].

The FTIR spectrum of NIF indicates the characteristic peaks of stretching vibration of some groups including N-H group at 3330.12 cm^{-1}, $-CH_3$ group at 2935.82 cm^{-1}, C=O group at 1684.47 cm^{-1}, C=C group (ring) at 1645.79 cm^{-1}, C-O-C group at 1118.22 and 1227.8 cm^{-1}. Besides, the peaks corresponding to bending vibration of NH, NO_2 and CH_3 groups are observed at 1538 cm^{-1}, 1529.01 cm^{-1}, and 1347 cm^{-1}, respectively [1].

In comparison with FTIR spectra of NIF and CS, It can be clearly seen a shift of absorption peak position of characteristic groups in CS and NIF in the FTIR spectrum of CS/NIF10 film.

The shift of peak of C-O, C=O and NH_2 group is 7 cm^{-1}, 93 cm^{-1}, and 96 cm^{-1}, respectively. This may be due to the formation of hydrogen bond and dipole-dipole interactions between CS and NIF in CS/NIF films as our suggestion in Figure 2.

The FTIR spectra of the CS/NIF30 and CS/NIF50 films are similar to CS/NIF10 spectrum so they have not been presented here.

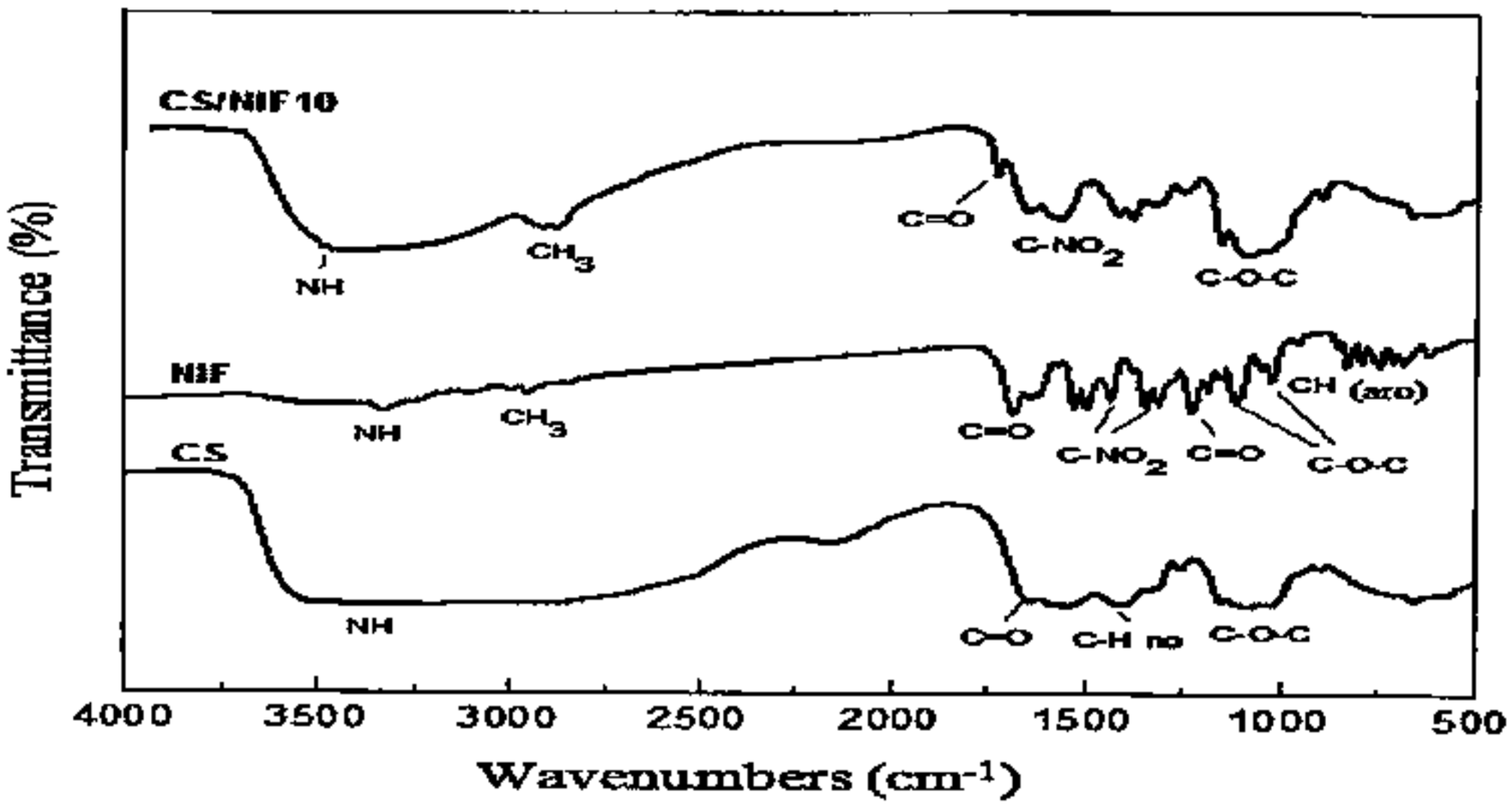

Figure 1. FTIR spectra of CS, NIF and CS/NIF10 film.

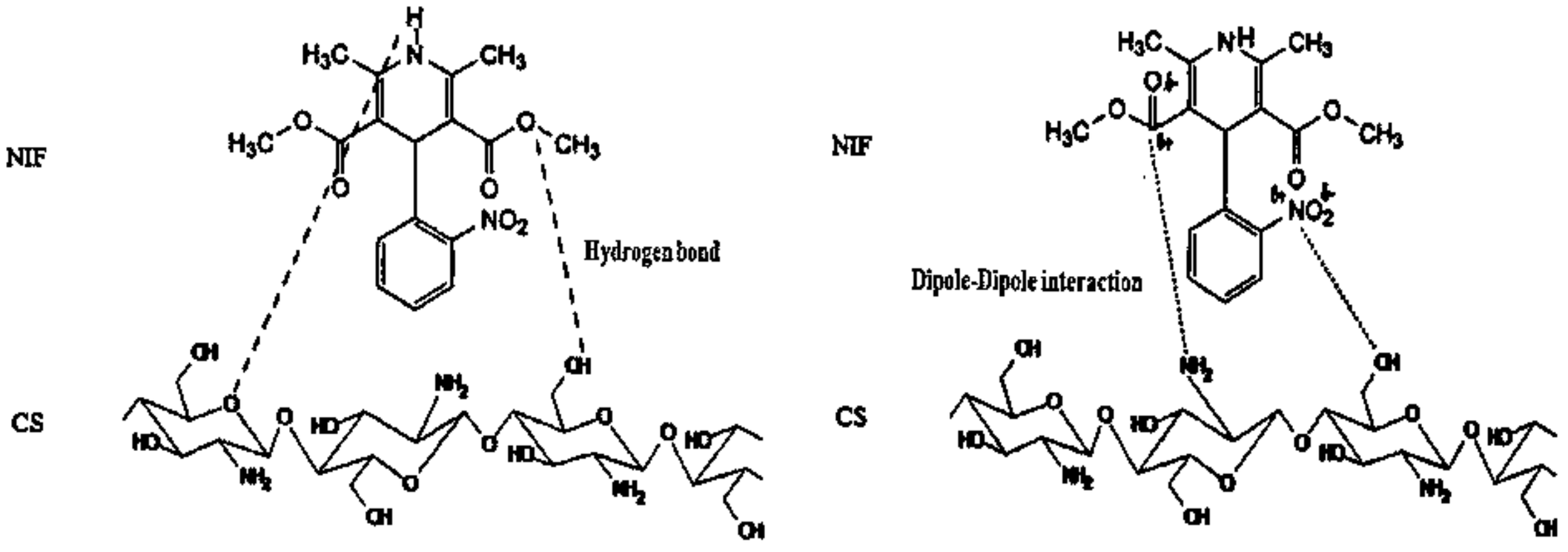

Figure 2. The suggestion of interactions between NIF and CS.

3.2. Morphology

Figure 3 presents FESEM images of CS, NIF and CS/NIF films. The FESEM image of neat CS displays quite uniform (Figure 3a). NIF has many different forms with basic size from 100 to 500 nm. The NIF agglomerates together by hydrogen interactions between NIF molecules leading to agglomeration size in the range of 2 to 5 μm (Figure 3b). For the CS/NIF films, NIF dispersed in CS matrix with small size from 200 nm to 1μm. The size of NIF in the matrix increases with raising NIF content in the films. At 50 wt% of NIF, NIF powders are agglomerated into the cluster with the size up to 2 μm in the matrix.

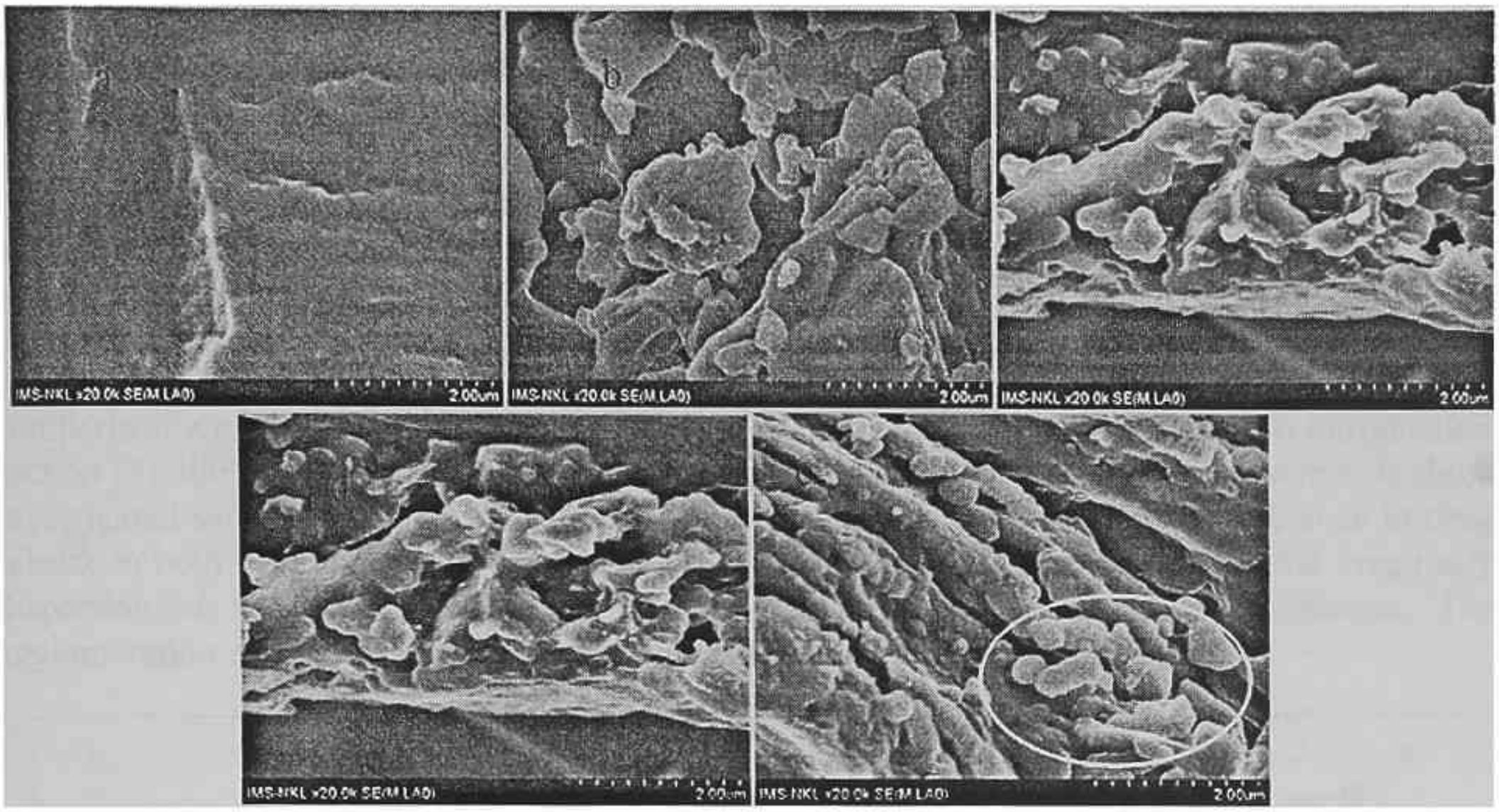

Figure 3. FESEM images of CS (a), NIF (b), CS/NIF10 (c), CS/NIF30 (d) and CS/NIF50 (e) films.

3.3. Thermal property

TG diagrams of CS and CS/NIF films are presented in Figure 4. The TG curve slope of CS and CS/NIF films is similar. The weight loss of CS/NIF films is higher than that of CS due to poor thermal stability of the NIF (the weight loss of NIF reached to approximate 90 % at 300 °C [12]). When increasing NIF content in CS/NIF films, weight loss of the samples also goes up.

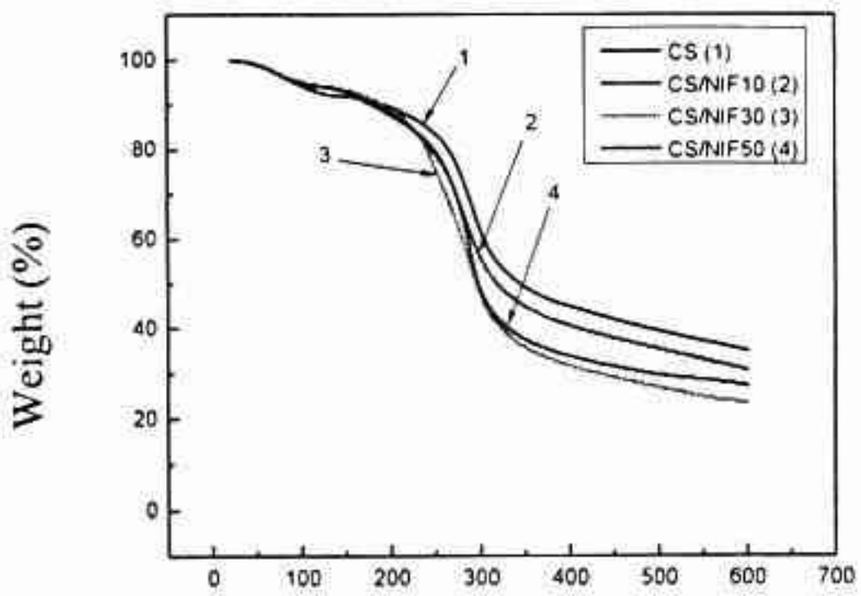

Figure 4. TG diagrams of CS and CS/NIF films.

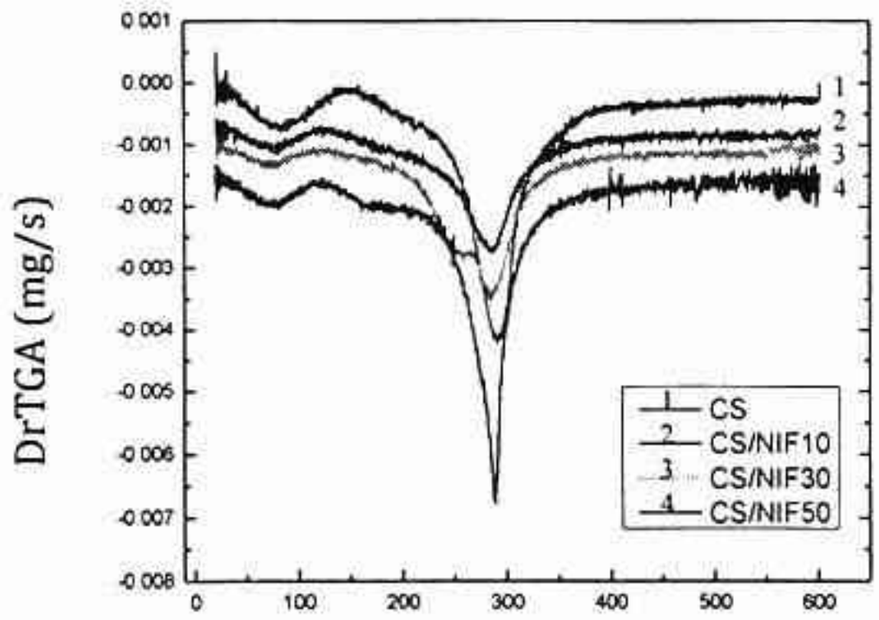

Figure 5. DTG diagrams of CS and CS/NIF films.

Figure 5 shows differential thermogravimetry (DTG) diagrams of CS and CS/NIF films and CS and CS/NIF films are degraded by a two-step process [13]. The first step occurring in the range of 90–250 °C corresponds to the loss of absorbed water, surface water and residual acetic acid. The second step is attributed to the decomposition of the saccharide circle, including

dehydration in the saccharide structure and the decomposition of acetyl and di-acetyl in chitin occurring in the temperature range of 250–500 °C, with maximum degradation temperature (T_{max}) at 289 to 291 °C (Table 1) [13, 14]. When adding NIF to CS, the onset temperature (T_{onset}) and T_{max} of CS/NIF films is lower than that of CS. It can be explained by the appearance of the defects and holes in the structure of CS/NIF films leading to the decrease in thermal stability of CS.

Table 1. Thermal characters of CS and CS/NIF films.

Sample	T_{onset} (°C)	T_{max} (°C)
CS	198.95	291.15
NIF	241.82	293.73
CS/NIF10	198.21	287.09
CS/NIF30	198.20	283.68
CS/NIF50	197.97	289.14

3.4. Drug release

The UV-VIS method is used to determine optical absorbance of the samples at wavenumber of maximum absorption. Based on values of recognized optical absorbance, the curves exhibiting the change of optical absorbance according to concentration of NIF in some different pH solutions (pH 7.4 corresponding to duodenum region in body where 90 % of the absorption of nutrients is taken in by the body and pH 6.8 corresponding to colon region in body where the waste products are passed out) are set up. The calibration equations and regression coefficients are calculated by Excel Software, in which x and y according to the optical absorbance (A) and concentration (C) of NIF and are shown in Table 2. The obtained results indicate that all obtained regression coefficients are higher than 0.996. Therefore, these calibration equations are applied to determine released NIF content from CS/NIF films at the different times.

Table 2. The calibration equations and regression coefficients (R^2) of NIF in pH 6.8 and 7.4 solutions.

No.	pH	λmax	Calibration equation	R^2
1	6.8	227	y = 29913x + 0.0189	0.9966
2	7.4	229	y = 17999x + 0.0114	0.9975

Figure 6 presents the NIF release percentage of the CS/NIF films (having 10, 30 and 50 wt% of NIF) immersed in pH 6.8 and pH 7.4 solutions. It is clear that both formulations gave a small different controlled release and NIF content released from CS/NIF samples increases slightly. Drug release curves of CS/NIF samples are nearly horizontal after 2 hours immersed in pH 6.8 solution and 5 hours in pH 7.4 solution. After 8 hour, 10, 30 and 50 wt% of NIF loaded samples released 50.22 %, 9.50 % and 9.52 % of NIF at pH 6.8 solution, respectively (Figure 6A). In pH 7.4 solution, the NIF release of these above samples after 8 hour was 44.55, 31.90 and 10.13, respectively (Figure 6B). The NIF release from polymer films is a rather complicated process. It can be affected by many factors such as polymer degradation, molecular weight,

crystallinity, the binding affinity between the polymer and the NIF and so on [15]. The NIF release rate is almost higher in pH 7.4 solution than pH 6.8 solution. The lower NIF release rate in the pH 6.8 solution (acidic pH) than the pH 7.4 solution (alkaline pH) is attributed to the repulsion between H^+ ions and cations on the surface of CS, which slow down the hydrolysis [15]. Compared with samples containing different NIF contents, it is clearly seen that NIF releases differently. CS/NIF10 sample has NIF release rate higher significantly than others in both tested pH solutions. This can be explained by the better dispersion of NIF in CS at 10 wt% NIF content as well as better NIF carrying ability of CS at this ratio (90/10). The purpose of the long-acting drug delivery systems is release rapid at first step and then is slow release. Thus, in comparison with NIF release ability of the patches containing NIF as mentioned in introduction section [9], the CS/NIF10 film is suitable to prepare long-acting drug delivery systems. In three investigated samples, CS/NIF10 and CS/NIF30 films exhibit the remarkable difference in drug release in both pH solutions unlike to CS/NIF50 film. This may be explained by the irregularly dispersion of NIF in CS matrix of CS/NIF50 film as seen at the FESEM images. The agglomeration of NIF in CS matrix can limit the diffusion of NIF into solution.

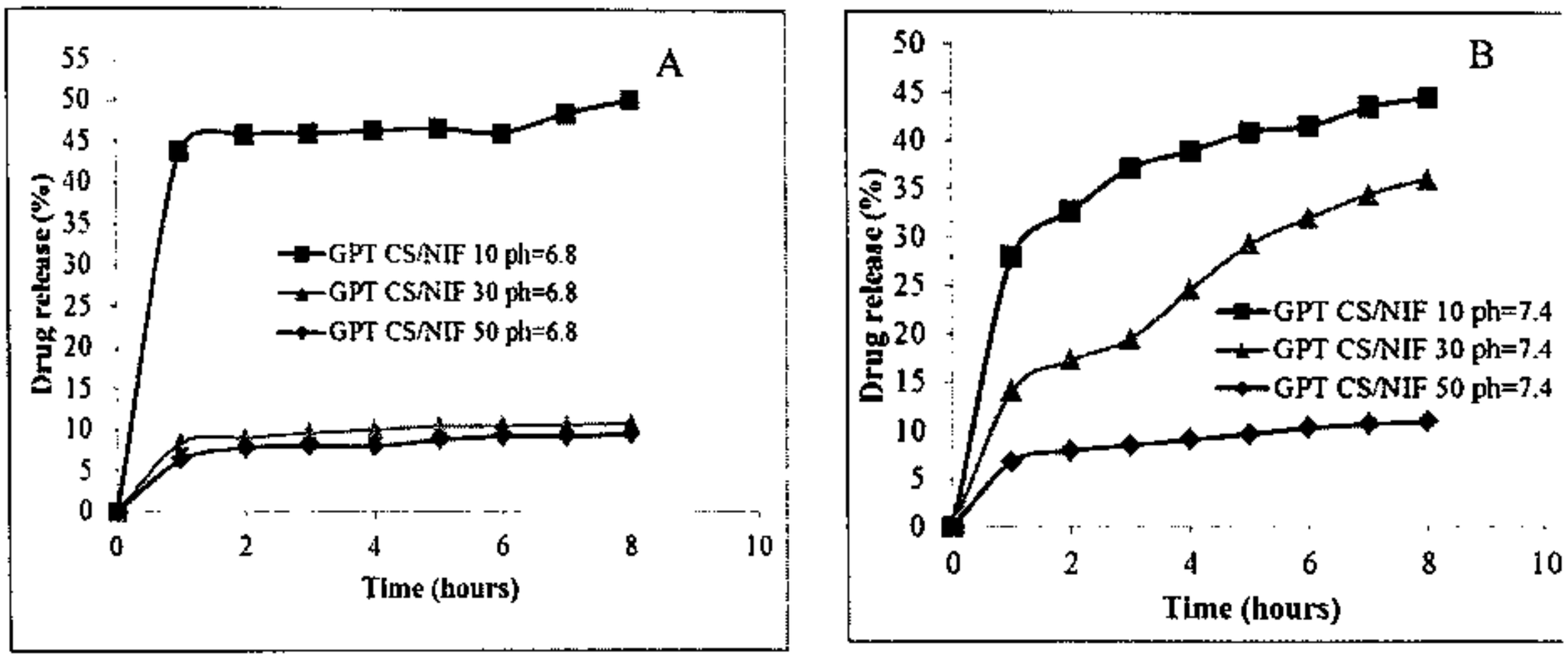

Figure 6. NIF release rate from different CS/NIF films immmersed in pH 6.8 (A) and pH 7.4 (B) solutions.

4. CONCLUSION

NIF powder disperses in the CS matrix with size in the range of 200 nm to 1 μm and the size of NIF in the matrix increases with raising NIF content from 10 to 50 wt%. CS and NIF are interacted together owing to hydrogen bond and dipole-dipole interactions between C-O, C=O, OH and NH groups in CS and NIF. Thermal stability of the CS/NIF films was smaller than that of the original CS.

The regression coefficients of the calibration equations exhibiting the change of optical absorbance according to concentration of NIF are higher than 0.996.

NIF release rate from CS/NIF films (containing 10, 30 and 50 wt% NIF) immersed in pH 7.4 solution is higher than that in pH 6.8 solution. CS/NIF10 has NIF release rate higher than others in both tested pH solutions.

Acknowledgments. This work has been financially supported by Vietnam National Foundation for Science and Technology Development (NAFOSTED, DT.NCCB-DHUD.2012-G/09) and Vietnam Academy of Science and Technology for Young Scientists 2015.

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TÓM TẮT

CHẾ TẠO, ĐẶC TRƯNG VÀ HÌNH THÁI CẤU TRÚC CỦA MÀNG CHITOSAN MANG NIFEDIPINE

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Bài báo này trình bày một số đặc trưng và hình thái của màng trên cơ sở chitosan (CS) và nifedipine (NIF) với các hàm lượng NIF khác nhau được chế tạo bằng phương pháp dung dịch. Phổ hồng ngoại biến đổi Fourier (FTIR) cho thấy các pic đặc trưng của một số nhóm nguyên tử trong CS và NIF dịch chuyển nhẹ so với các pic tương ứng trên phổ FTIR của CS và NIF. Điều này chứng tỏ CS và NIF đã tương tác với nhau. NIF phân tán trong nền CS với kích thước hạt tương đối nhỏ, từ 200 nm đến 2 µm. Kết quả phân tích nhiệt chỉ ra màng CS/NIF kém bền nhiệt hơn CS. Phương trình đường chuẩn của NIF được thiết lập từ dữ liệu phổ UV-Vis đều có hệ số hồi quy lớn hơn 0,996. Nghiên cứu giải phóng thuốc của màng CS/NIF cho thấy tốc độ giải phóng thuốc ở pH 7,4 lớn hơn ở pH 6,8 cho tất cả các mẫu chứa 10, 30 và 50 %KL NIF.

Từ khóa: chitosan (CS), nifedipin (NIF), tính chất nhiệt, phương pháp dung dịch, giải phóng thuốc.