Preparation of Shitosan/ Montmorillonite (MMt) Nanocomposite as a Drug Delivery Carrier of Podophyllotoxin

Soheila Sedaghat

Department of Chemistry, Islamic Azad University,
North Tehran Branch, Tehran, Iran

Email: s_sedaghat [AT] iau-tnb.ac.ir

ABSTRACT--- ***Biopolymer composites have chosen for carrying drugs because they decrease the side effect of drug and enhance the appropriate concentration in the treatment sites. The biocompatibility and nontoxicity of biopolymers is a main reason to apply them in pharmaceutical fields. The aim of this work was to develop a biopolymer composite based on chitosan (CTS) and Montmorillonite (MMt) by a simple cross-linking reaction using glutaraldehyde as the cross-linker. The swelling ratio in two pH solutions, drug encapsulation efficiency and controlled release behavior were investigated by using Podophyllotoxin as a drug. The results show that the incorporation of MMt improved the swelling behavior, enhanced the drug entrapment efficiency, and decrease the drug release rate. The exfoliated clay could act as a physical cross-linker to facilitate the formation of network structure between the CTS and MMt. It is determined that MMt may be developed as an effective additive for fabricating a sustained drug delivery system.***

Keywords--- Chitosan, Podophyllotoxin, nanocomposite, release rate, montmorillonite (MMt), drug delivery carrier, Thermal analysis

1. INTRODUCTION

Chitosan (CTS) , a polysaccharide consists of linear chain of D- glucosamine and N- Acetyl-D- glucosamine units(Fig.1). It is prepared by deacetylation of chitin (abundant natural polysaccharide found in the exo-skeleton of crustaceans such as crabs and shrimp). Depending on conditions of the deacetylation process, different forms of chitosan are available. Those forms vary in the deacetylation degree (DD) and the average molecular weight (Mw) of the polymer [1,2]. Both of those features, determine the physicochemical properties of the polymer and its application[3,4,5]. Chitosan is a polymer dissolved in dilute acid solutions and form a network of polymer chains by name hydrogel. They have the ability to sense changes of media acidity [6] or temperature and release their load as result of such a change [7]. The biocompatibility, biodegradability and swellability properties of chitosan polymer have been considered in the drug delivery carrier [8,9].

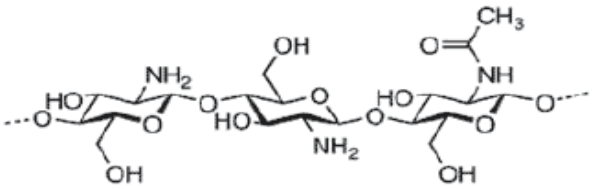


Fig. 1. Chitosan's structure

Chemical cross-linking is a straightforward method to produce permanent hydrogel networks using covalent bonding between polymer chains [10]. These networks can be formed by using small molecule cross-linkers, polymer–polymer reactions between activated functional groups [11], as well as photosensitive agents or enzyme-catalyzed reactions [12,13]. Cross-linked chitosan networks can be prepared by covalent bond formation between amine functional group in chitosan chain and aldehyde group from cross-linkers molecule like glutaraldehyde(Fig.2).

Clay minerals have been used as excipients and active agents for the development of new drug delivery systems. Layered silicates as a drug delivery carrier have been used because their unique layer structure can accommodate polar organic molecules to form various intercalated compounds [14].The layer structure of the silicate can provide a large space to reserve neutral drug molecules via ion-dipole interaction and cationic or bio functional via ion exchange reaction. The idea is to store the drug in the interlayer region of the lamellar host and allow the drug release as a consequence of diffusion and/or de-intercalation process [15].

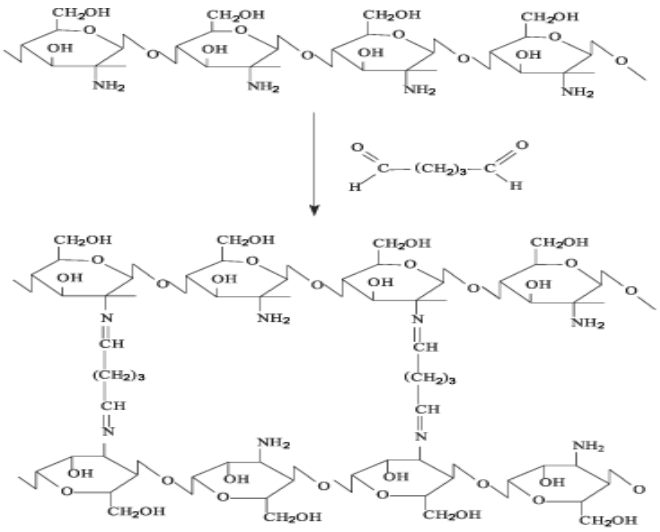


Figure 2. Crosslinking process of chitosan with glutaraldehyde

Montmorrilonite(MMt) is a plate-like synthetic hectorite-type clay which belongs to a family of phyllosolicates (trimorphic) . It has a Large surface area, anionic surface charges and exchangeable Na+ cations in hydrated interlayers, and therefore it shows better adsorption properties for cationic drug molecules .Many kinds of composites based on MMt were prepared and evaluated, e.g., MMt -itraconazole nanohybrid [16,17], and modified poly(vinyl alcohol)/ MMt nanocomposite membrane [18]. It was found that the exfoliated MMt particles may act as multifunctional cross-linkers in forming the composite hydrogels, and the polymer chains were anchored to the particles and entangled to form a network [19]. MMt has negative surface charges and exchangeable cations which can form strong interfacial interactions with cationic drug and show relatively higher absorption value than other types of clays.It is expected that the introduction of MMt can form new types of composite hydrogels with improved mechanical properties, swelling behaviour, drug loading efficiency and controlled release behaviour. The CTS/montmorillonite (MMt) nano enhances the stability of the film and exhibited good potential for the use as drug carriers for sustained release [20].

Podophyllotoxin(PTX), as a lignin family (Fig.3), is an antimitotic material (anti-mitosis and cell proliferation) and impairs cell proliferation by binding to tubulin proteins in the mitotic spindles [21]. Our group has been working during the last few years on tissue culture synthesis of podophyllotoxin and its glycosyl analog. [22] Due to these biological activities, they have been the objective of numerous studies focused to prepare better and safer anticancer drugs and in cancer chemotherapy. The main deficiency is their cytotoxicity for normal cells and hence side effects derived from their lack of selectivity against tumor cells. In this regard it is necessary to investigate and prepare a delivery method.

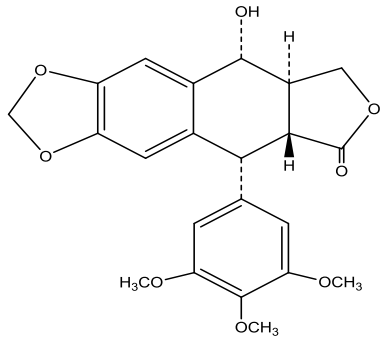


Figure 3-the structure of podophyllotoxin

Based on this background, as a part of the systematic work of designing new type of composite drug delivery carrier, a composite hydrogel of CTS/ MMt film were prepared, and the interaction between MMt and CTS and the influence of MMt on the entrapment efficiency, swelling ratio and drug release properties of the matrices were evaluated. The swelling ratio and in vitro drug release profiles were tested in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4) using Podophyllotoxin as a drug candidate. The structure and morphology of the film were characterized by FTIR, XRD and SEM techniques.

2. EXPERIMENTAL

Materials :

Chitosan (CTS, deacetylation degree 81%; weight average molecular weight) Aldrich (EC 222-33-2) . Podophyllotoxin(PTX), medical type was obtained from pars azma co. IRAN. Montmorillonite (MMt, 99%), Cloisite 30B was obtained from Southern Clay USA**.** Acetic acid (Merck), glutaraldehyde (GERBU Biotechnik GmbH). Simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4) were prepared in terms of US Pharmacopeias. All other chemicals were of analytical grade and used as received. *This article does not contain any studies with human participants performed by any of the authors.*

X-ray diffraction (XRD), Bruker D8 Advance diffractometer, Germany, CuKά radiation, Nickel filter, HPLC (WatersTM 600 Pump, 2998 Photodiode Array Detector, USA), UV-spectrophotometer

Methods :

Chitosan was prepared in 1% aqueous acetic acid at room temperature and was kept at room temperature for overnight in the shaker to obtain a (1%w/v) solution. We added glutaraldehyde by 1:0.2 molar ratio [23] as cross liking agent and the resulted uniform solution dryed at room temperature for 3 days. A yellow uniform hydrogel film was formed and we measured the uniformity of film tickness.

The same method has been used to prepare a polymer film containing podophyllotoxin. To prepare the drug solution about 200mg of PTX drug was dissolved in minimum amount of ethanol; the solution was stirred until a homogenous mixture is formed. PTX solution was gradually added to the CTS solution before the crosslinked agent adding with continuous stirring. The mixture was then stirred for further 24 hours at room temperature (20°C). CTS-PTX Films were prepared by pouring and spreading the CTS-PTX mixture on a glass plate, and kept them for two days .

Montmorillonite/chitosan composite: The suitable amounts of Montmorillonite (0, 0.20, 0.40, 0.60 and 0.80 g) were each dispersed in 20 mL of 2% (v/v) acetic acid-ethanol solution, and then 0.20 g podophyllotoxin was added to each dispersion and all mixtures were stirred at room temperature for 1 h. Subsequently, chitosan powder (0.60 g) was dissolved directly into the mixture solution to a final concentration of 3% (w/v) under continuous stirring for 1 h. We added glutaraldehyde by 1:0.2 molar ratio and the obtained gel-like material was firstly air-dried for 24 h and then oven-dried at 70°C for 6 h.

Swelling ratio: Each piece of films were designed in 2.2 × 2.9 cm .The pieces were floated in 100 ml of distilled water at room temperature and stirred up to become swell completely. At the time intervals of 30, 60, 120,240 and 420 minutes, the films were taken out and excess water was removed from the film carefully using filter paper and films were weighed immediately. The amount of swelling or swelling ratio was calculated using the following formula:

$$100 \times [(W_t - W_0) / W_0] = \text{Swelling ratio}$$

In the formula ,W_t and W_0 are the weights of the samples in the dry and swollen states, respectively. We calculated the temperature effect on swelling ratio. In order to check the swelling ability of the hydrogel in two acidity media. The films (1.5×1.5 cm2) were placed in 50 ml of two pH solution ,1.2 and 7.4 .

Drug release rate: In order to prepare the standard curve of podophyllotoxin concentration , first , the solution of 0.2 g/lit of Podophyllotoxin in ethanol was prepared, and then divided it to the solutions with different concentrations. The absorbance of each solution was measured by the spectrophotometer.

Each pieces of films were designed in 2.2 × 2.9 cm and environ has 2 mg of podophyllotoxin . The pieces was floated in 100 ml of distilled water at room temperature and stirred up to become swell completely. Same volume of samples was taken at the time intervals of 30, 60, 120,240 and 420 minutes,and their absorbance was measured using the spectrophotometer.

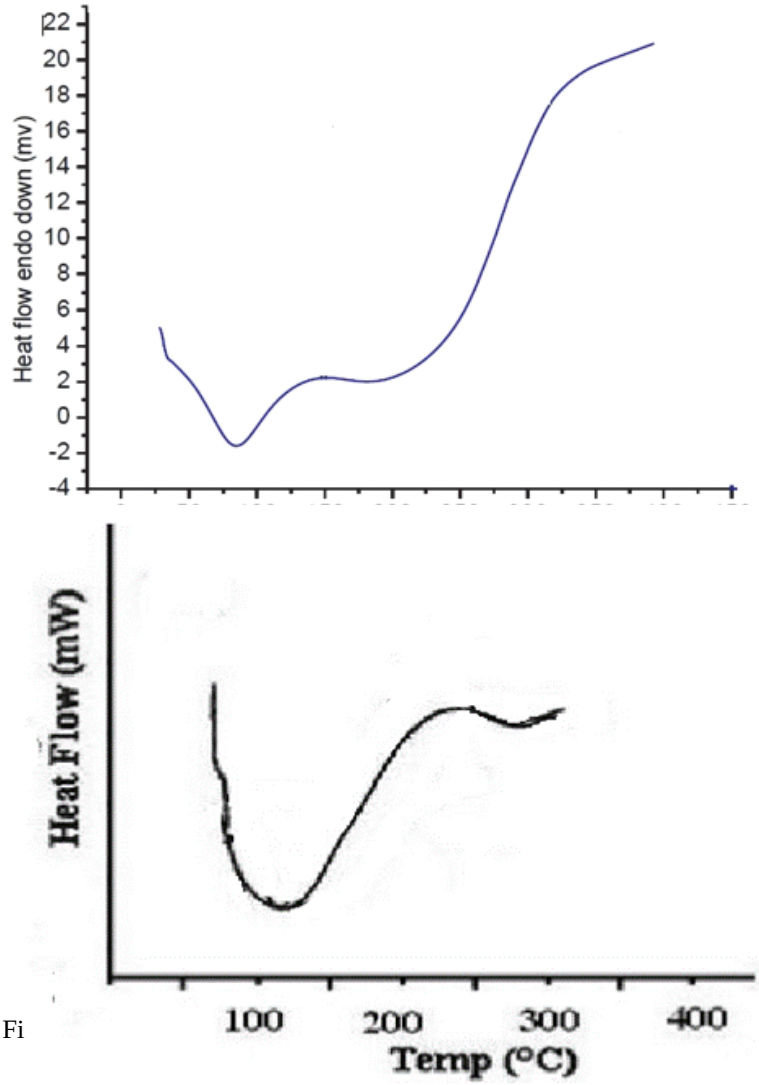
Entrapment Efficiency Entrapment efficiency (EE) is one of the critical parameters that have to be considered in the preparation of drug-loaded hydrogel. In this study, we determined the loss of drug during the entire manufacturing processes. In addition, with an emphasis on assessing the stability of drug during the preparation process and determining the amount of drug in the prepared film simultaneously, the HPLC method was selected for entrapment efficiency test. The procedure adopted was as follows: after preparing the film, the supernatant was collected and diluted with distilled water, then filtrated through membrane filter to remove the floating tiny amount of Montmorillonite particles. The clear superficial solution was analyzed by HPLC. The drug content was determined by comparing with the standard curve of the drug encapsulation efficiency is expressed as follows:

$$Entrapment\ efficiency\ (\%) = \frac{Practical\ drug\ loading}{Theoretical\ drug\ loading} \times 100$$

3. RESULTS AND DISCUSSION

Chitosan /MMt nanocomposites have shown potential for the drug delivery carrier because they can increase the drug utilization and enhance the controllable capability of drug release. Furthermore, the biocompatibility and nontoxicity of biopolymers is retained, and therefore it is promising and applicable in pharmaceutical fields. The physical properties of a hydrogel, such as swelling ratio and drug release rate can be improved by controlling the GA concentration[?].The montmorillonite in the chitosan nanocomposite could form a porous surface and improve water absorbence.

The DSC analysis of chitosan /montmorillonite nanocomposite (Fig.4) was shown following ; two broad endothermic peaks at 92.3 ºC and 212 ºC. The former peak may be due to the water vapor present in CTS while the latter may be attributed to the molecular arrangement of CTS chains. The CTS-PTX complex exhibit (Fig.5) a sharp endothermic peak at 120ºC due to structural arrangement. There is a sharp exothermic peak at 238ºC is due to the thermal decomposition of PTX.



Fi

Figure6 shows the FTIR spectra of CTS, Podophyllotoxin and MMt samples. It can be seen that the characteristic bands of CTS at 1651 and 1602 cm^{-1} (vibration bands of amide I and -NH_2, respectively) shifted to 1640 and 1557 cm^{-1} after cross-linking with glutaraldehyde, indicating that these groups interacted with GA. The absorption band of MMt at 3627 cm^{-1} (stretching vibration of the (Si) O-H groups) [16] cannot be quite observed after forming composite film due to the reduction of MMt content, but the Si-O(H) characteristic stretching vibration of MMt at 1016 cm^{-1} clearly appears in the spectrum of the composite which has shifted to 1013 cm^{-1} [24]. This indicates that MMt existing in the film has formed a composite structure with CTS and GL. The characteristic absorption bands of MMt have shifted to low wave number region, revealing that the electrostatic and hydrogen bonding interactions have taken place among the silanol groups (-SiO^-) of the clay and the - NH_3^+ groups of CTS [25]. The introduction of MMt has led to a change in matrix composition of the film. The C=O absorption bands at 1714 cm^{-1} (-COOH groups) and at 1623 cm^{-1} (C=O group of Podophyllotoxin)

appeared in the spectrum, but shifted to 1707 cm^{-1} and 1625 cm^{-1}, respectively. This result indicates that the Podophyllotoxin drug was loaded on the composite hydrogel film and the positively charged Podophyllotoxin combined with the negatively charged surface of MMt like a cationic dye adsorption mechanism [26].

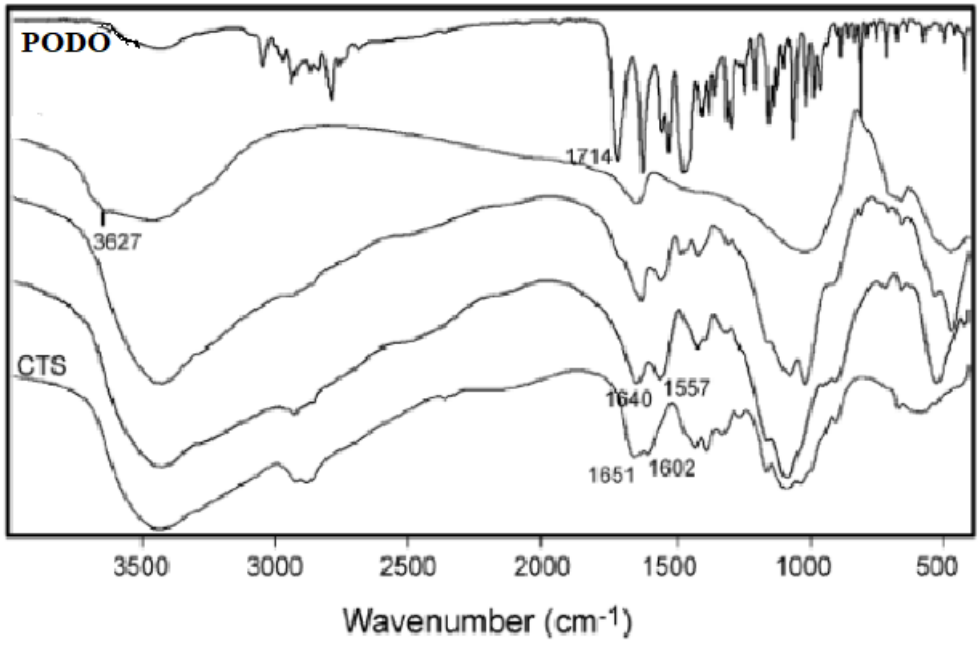


Fig 6 - FTIR spectra of chitosan /montmorillonite nanocomposite

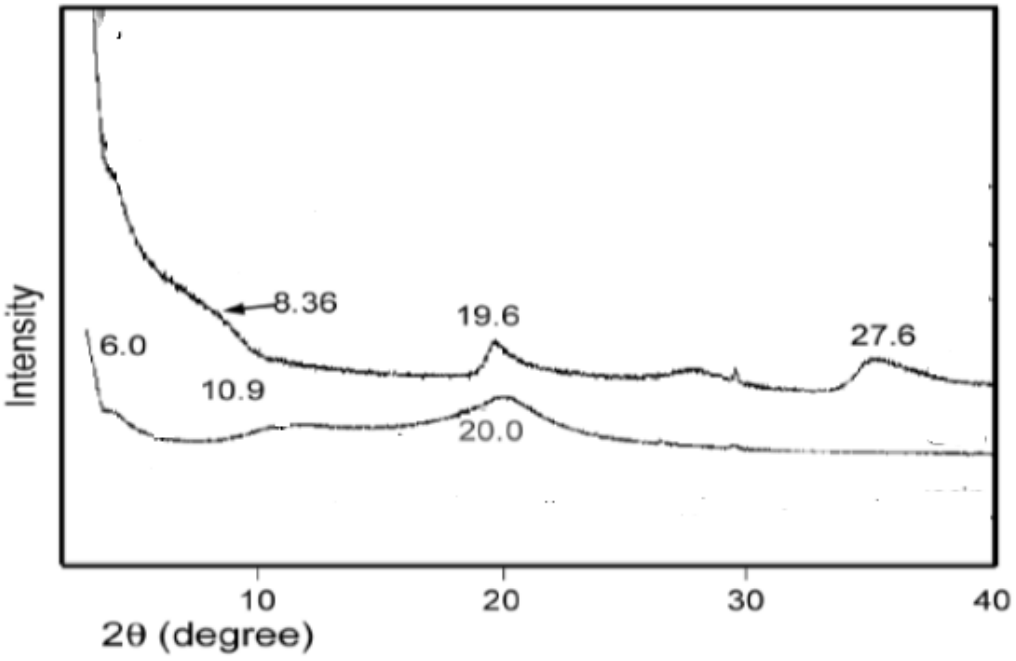
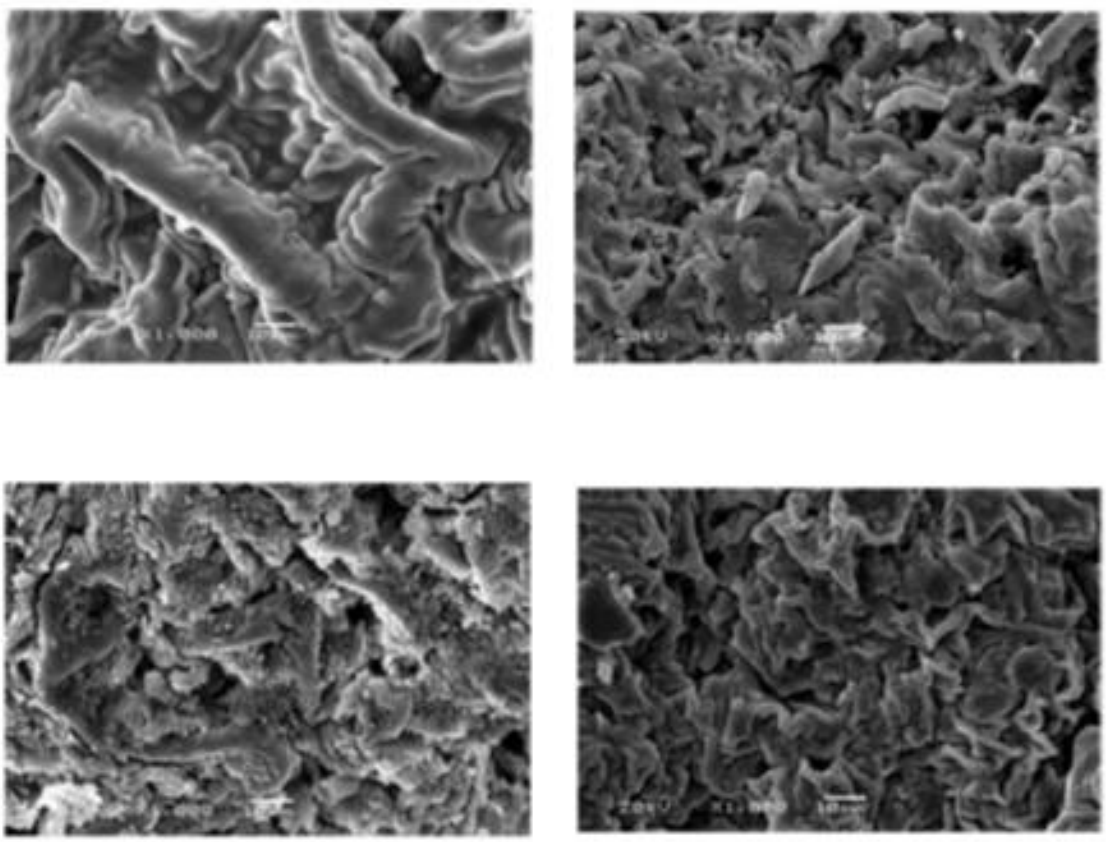


Fig 7. X-ray diffraction (XRD) of chitosan /montmorillonite nanocomposite

X-ray diffraction (XRD) studies (Fig 7) were performed to determine crystalline patterns of the drug in the cross-linked polymer network. The XRD patterns from 3° to 40° exhibit the crystal peaks of drug-loaded chitosan and blank composite. One typical peak near 20° can be observed for CTS, but its diffraction intensity has weakened drastically in the sample. The diffraction peaks of MMt at 19.6° and 27.6° appear in the composite, indicating the incorporation of MMt and formation of composite structure. Although the entrapment of Podophyllotoxin has been proved by infrared

spectra, UV-Vis measurement and drug release experiment, the diffraction peaks of Podophyllotoxin cannot be observed in the XRD pattern of sample.

Detailed examination of the surface structure by SEM (Fig.8) reveals servale wrinkles caused by partial collapsing of the polymer network during drying which are in agreement with other research findings [27]. The addition of drug imparts a high degree of surface roughness which may be due to crystals leaking from the film network structure [28].



Swelling Ratio

The release of any encapsulated drug from chitosan /montmorillonite nanocomposite film requires a re-hydration process. For this reason, swelling experiments of representative formulations were carried out in pH 1.2 solutions at 37°C(Fig.9) . It is a general observation that at an acidic pH, the swelling ratio of the film is high .The sample was found to dissolve completely at pH 1.2 within 3 h of immersion, whereas it was swollen with its weight increasing by 0.3 times within 30 min in pH 1.2 solution. Thus, the CTS film without any MMt were stable only in pH 1.2 solution.

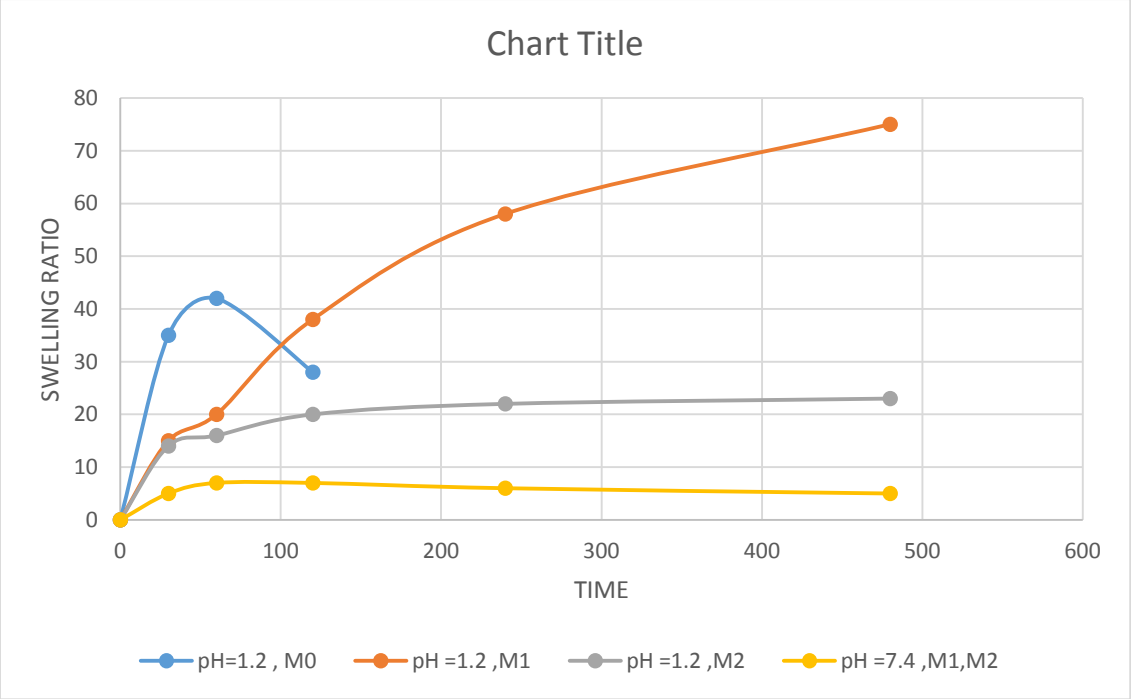


Fig.9 Swelling data in pH 1.2 solutions at 37°C

Furthermore, with higher MMt content, the initial swelling rate at pH 1.2 is decreased and the swelling ratio within 4 h is increased significantly. The film are highly swollen at gastric pH (1.2). This high swelling ratio is attributed to the electrostatic repulsive force resulting from the positive charge of the protonated amine groups of CTS, an indication that these groups are mainly responsible in pH-sensitive swelling capacity. If the cross-linking density is too low, the interactions are no longer strong enough to avoid disintegration, and therefore the film disappear completely within 2 h. The stronger gel matrix and lower disintegration resulting from the introduction of MMt is due to the dispersion of clay platelets in an aqueous medium which could act as an effective multifunctional cross-linking agent [29]. As shown in Figure 9, the presence of MMt increases the degree of crosslinking which it decreases the degradation rate of the film.

Drug release rate

The release rate of Podophyllotoxin from the film was measured in both pH 1.2, pH 7.4, solutions at 37°C. As shown in Figure 10 , almost the total Podophyllotoxin loaded in first sample was released within the initial 2 h following its incubation in pH 1.2. This may be attributed to the low matrix stability and the higher drug solubility at this pH environment [30].

The drug release pattern at pH 7.4 showed an initial burst and slow release which is followed by sustained release (Figure 11) . The initial burst release might be the result of the rapid dissolution of the drugs and the subsequent sustained release might be the dominant release mechanism, which changed to drug diffusion through the CTS matrix.

The effect of incorporation of MMt layers can be significantly observed in reduced rate of release. The explanation of this behaviour is the greater cross-linking density with greater MMt content as well as the existence of strong electrostatic interaction between the protonated amino groups of Podophyllotoxin cations and the anionic groups of the layers of MMt which are not overcome by the interaction with the solvent.

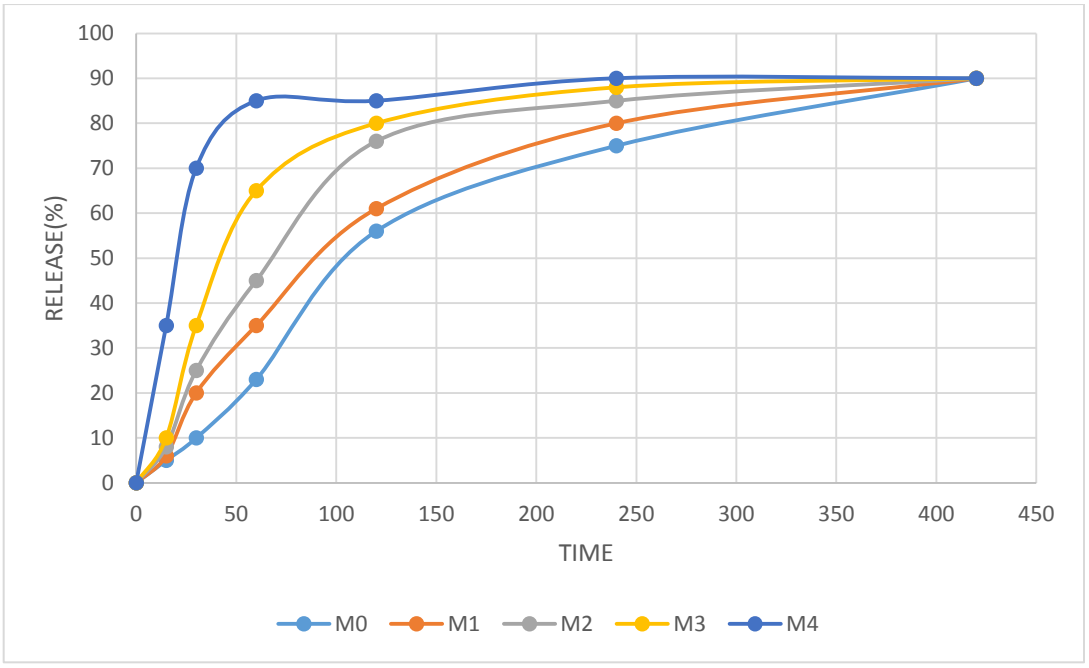


Fig.10 –drug release rate at pH 1.2

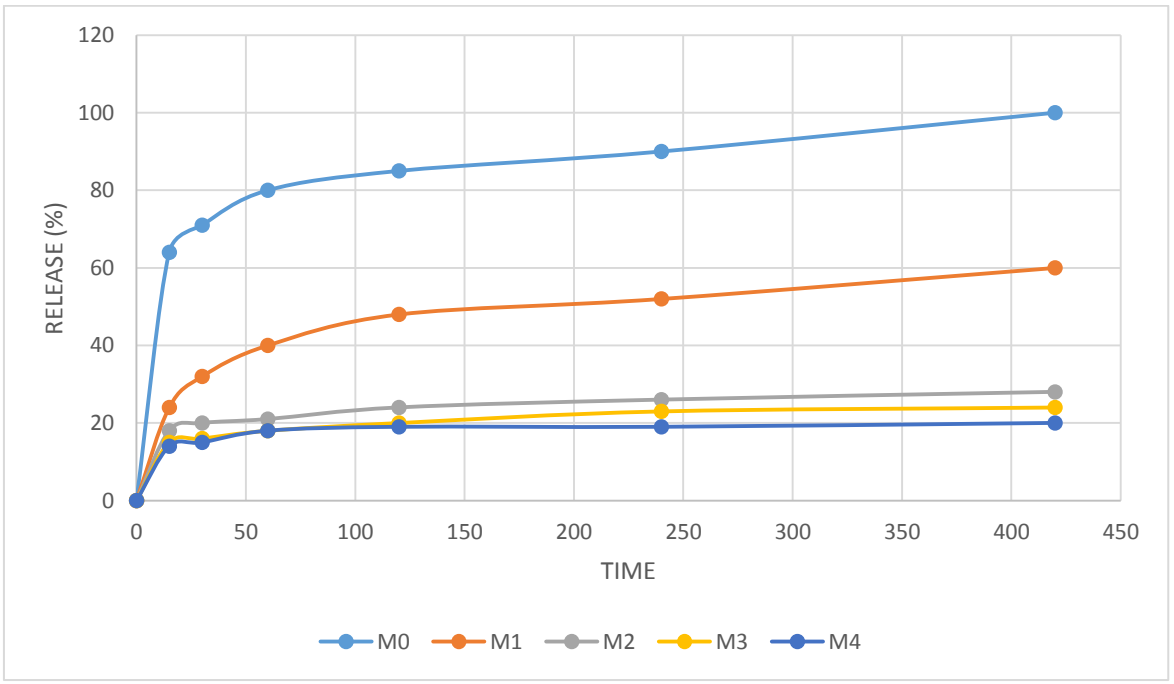


Fig.11 –drug release rate at pH 7.4

Kinetic data can be applied to investigate the release rate of the podophyllotoxin compound from hydrogel. The concentration changes based on time showed that the release rate of the podophyllotoxin is following the first order rate equation.The variable data obtained from the study is plotted versus the time and it is observed that the graph versus time is linear. Drug release profile of formulations containing chitosan followed the first order. The resulting pH-sensitive network of matrix facilitated sustained release of drug.
the release of drug from polymer is due to Fickian diffusion.

For detecting the entrapment efficiency, there was a single peak representing Podophyllotoxin in the chromatogram, suggesting that the molecule was stable during preparation of the film. The entrapment efficiency of the composite film for Podophyllotoxin increases with the increased MMt content in the range of 28.5 % to 89.9 % (Table 1).

Table 1. Entrapment efficiency data

Sample	Entrapment efficiency
M0	**28.5**
M1	**45.9**
M2	**62.3**
M3	**75.5**
M4	**89.9**

From the characterization results of the swelling and in vitro release studies, a possible model for the composite film is shown in Figure 12 , where the cross-linking between CTS and GL and electrostatic interactions between CTS and MMt as well as Podophyllotoxin are indicated. It is worth to note that the addition of a certain amount of MMt not only improves the drug entrapment efficiency of the composite film, but also provides a slower and sustained drug release. Thus, the inclusion of MMt clay into biopolymers may be used for the sustained delivery of drug and other bioactive molecules.

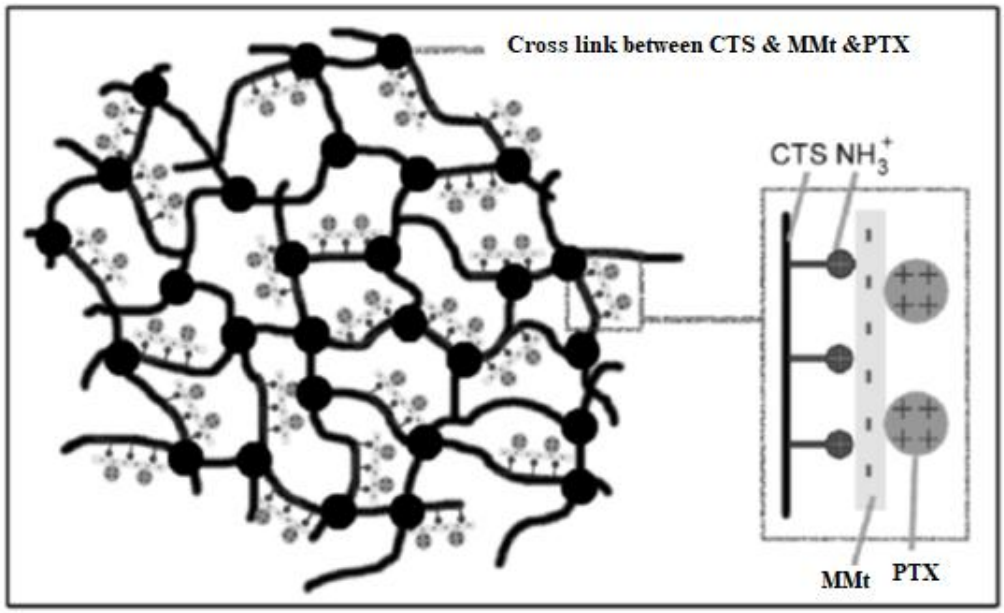


Fig.12- model for the composite film

4. CONCLUSION

In summary, the intercalated CTS/MMt composite samples were developed as a novel drug delivery carrier. Molecular interaction of CTS with MMt caused a change of the characteristics of the composite film. The interfacial interactions between CTS and MMt via electrostatic interaction could improve the swelling behaviour, drug loading efficiency and controlled release rate. The disintegration of the pure CTS film at pH 1.2 was overcome by introduction of MMt which made this system more useful in targeting lower acidity.

The herbal medicine compound of Podophyllotoxin has harmful side effects. This drug causes side effects such as hair loss of patient and other side effects including loss of appetite, nausea and vomiting. In order to facilitate the use of this drug in treating the disease, it seems that using the controlled drug release system is necessary. Chitosan hydrogel structure was used as the controlled drug delivery system to combine Podophyllotoxin. The release rate of Podophyllotoxin was obviously lowered with increased MMt content. Therefore, the introduced MMt endowed the composite film with a controllable degradation rate.

5. REFERENCES

1. Fernandez , J ., longber , D .," Manufacturing of Large-Scale Functional Objects Using Biodegradable Chitosan Bioplastic", Macromol . Mater . eng., 299 , 932 -938 ,2014
2. M. Dash , F. Chiellini , R.M. Ottenbrite , E. Chiellini ," Chitosan—A versatile semi-synthetic polymer in biomedical applications ", Progress in Polymer Science 36 ,981–1014,2011
3. Hejazi , R ., Amiji , M ., "Chitosan-based gastrointestinal delivery systems " , Journal of Controlled Release . ,89 , 151 -165 ,2003
4. Friedman A.J., Phan J., Schairer D.O., Champer J., Qin M., Pirouz A., Blecher-Paz K. , Oren A. , Liu P.T., Modlin R.L., Kim J. ," Antimicrobial and anti-inflammatory activity of chitosan-alginate nanoparticles: a targeted therapy for cutaneous pathogens", Journal of Investigative Dermatology ,133, 1231–1239, 2013
5. Shakeel A., Saiqa I., "Chitosan Based Scaffolds and Their Applications in Wound Healing", Achievements in the Life Sciences 10 ,27–37 ,2016
6. Shivashankar M.,K Mandal B. ," Design and Evaluation of Chitosan-Based Novel pH Sensitive Drug Carrier for Sustained Release of Cefixime ", Tropical Journal of Pharmaceutical Research, 12,2,155-161,2013
7. Bhattarai N. , Gunn J., Zhang M., " Chitosan-based hydrogels for controlled, localized drug delivery ", Advanced Drug Delivery Reviews 62 , 83–99,2010
8. Semwal A., Ramandeep S.," Preparation, characterization and biological evaluation of chitosan/moxifloxacin prodrugs for pharmaceuticals ", Marmara Pharmaceutical Journal 18: 36-42, 2014
9. Khoee S., Kardani M., "Hydrogels as Controlled Drug Delivery Carriers", Polymerization, Quarterly,2,4, 16-27,2013
10. Kumar Giri , T ., Thankur , A ., Alexander , A ., Badwaik , H ., Tripathi , D ., "Modified chitosan hydrogels as drug delivery and tissue engineering systems: present status and applications", Acta pharmaceutic , Sini ca . ,2 , 439-449,2012
11. Mirzaie , E. , Ramazani , A. , Shafaiee , M. , Danaei , M., "Studies on Glutaraldehyde Cross-linked Chitosan Hydrogel Properties For Drug Delivery Systems ", International Journal Of Polymeric Materials and Polymeric Biomaterials .,62 ,.605 -611,2012
12. Saifuddin N., yasumira N.," Microwave Enhanced Synthesis of Chitosan-graft- Polyacrylamide Molecularly Imprinted Polymer (MIP) for Selective Extraction of Antioxidants" , E-Journal of Chemistry ,7,4, 1362-1374,2010
13. Abdollahi paynavandi M., Ebrahimi R., Amiri A ., " Study of Loading and Releasing of Fluvoxamine in Hydrogels Prepared by Ultrasound Irradiation", Iranian Journal of Polymer Science and Technology 28,3, 225-232 , 2015
14. Viseras C, Cerezo P, Sanchez R, Salcedo I, Aguzzi C," Current challenges in clay minerals for drug delivery", Appl Clay Sci, 48, 291-295, 2010.
15. Depan D, Kumar AP, Singh RP, " Cell proliferation and controlled drug release studies of nano hybrids based on chitosan-g-lactic acid and montmorillonite" , Acta Biomater, 5, 93-100, 2009.
16. Jung H, Kim HM, Choy YB, Hwang SJ, Choy JH," Itraconazole-laponite: kinetics and mechanism of drug release", *Appl Clay Sci*, 40, 99-107, 2008.
17. Jung H, Kim HM, Choy YB, Hwang SJ, Choy JH, "Laponite-based nanohybrid for enhanced solubility and controlled release of itraconazole", *Int J Pharm*, 349, 283-290, 2008.
18. Nair SH, Pawar KC, Jog JP, Badiger MV, "Swelling and mechanical behavior of modified poly (vinyl alcohol)/laponite nanocomposite membranes", *J Appl Polym Sci*, 103, 2896- 2903, 2007.
19. Mujumdar, SK, Siegel RA, ;'Introduction of pH sensitivity into mechanically strong nanoclay composite hydrogels based on N-isopropylacrylamide" , J Polym Sci Pol Chem, 46, 6630- 6640, 2008.
20. Hua SB, Yang HX, Wang WB, Wang AQ, "Controlled release of ofloxacin from chitosan/montmorillonite hydrogel" , Appl Clay Sci, 50, 112-117, 2010.
21. Wei-Hua Cheng, Hai Shang , Cong Niu , Zhong-Heng Zhang , Li-Ming Zhang , Hong Chen , Zhong-Mei Zou , " Synthesis and Evaluation of New Podophyllotoxin Derivatives with in Vitro Anticancer Activity" , Molecules , 20, 12266-12279,2015
22. Sedaghat S., Ezatzadeh E.,Alfermann A.W. ,Natural Product Research,1, 6 ,2007
23. Krishna Rao KSV, Rao KM, Kumar PVN, Chung I-D, " Novel chitosan-based pH sensitive micronetworks for the controlled release of 5-fluorouracil" , Iran Polym J, 19, 265-276, 2010.
24. Li P, Kim NH, Siddaramaiah, Lee JH, " Swelling behavior of polyacrylamide/Laponite clay nanocomposite hydrogels: pH-sensitive property" , *Compos B Eng*, 40, 275-283, 2009.
25. Khunawattanakul W, Puttipipatkhachom S, Rades T, Pongjanyakul T, "Chitosan-magnesium aluminum silicate composite dispersions: characterization of rheology, flocculate size and zeta potential", *Int J Pharm*, 351, 227-235, 2008.
26. Li P, Siddaramaiah, Kim NH, Heo S-B, Lee J-H, " Novel PAAm/Laponite clay nanocomposite hydrogels with improved cationic dye adsorption behavior" , *Compos B Eng*, 39, 756-763, 2008.

27. Srinatha A, Pandit JK, Singh S, "Ionic crosslinked chitosan beads for extended release of Ciprofloxacin: In vitro characterization", Indian J Pharm Sci, ,70, 16-21, 2008.
28. Prabaharan M, Reis RL, Mano JF, "Carboxymethyl chitosan-graft-phos phatidyl ethanol amine: amphiphilic matrices for controlled drug delivery", React Funct Polym, 67, 43-52, 2007
29. Zhang QS, Zha LS, Ma JH, Liang BR, "A novel route to the preparation of poly (*N*-isopropylacrylamide) microgels by using inorganic clay as a cross-linker" , *Macromol Rapid Comm*, 28, 116- 120, 2007
30. Cui Y, Zhang Y, Tang X, "In vitro and in vivo evaluation of ofloxacin sustained release pellets", Int J Pharm, 360, 47-52, 2008.