PAPER

Thermal sensitive Poloxamer/Chitosan hydrogel for drug delivery in vagina

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Abstract

In this paper, the composite gels based on poloxamer and chitosan with different contents were prepared for thermal sensitive mucosal drug delivery. The sol-gel transition temperature and rheological viscosity of the obtained gels were detected to select the optimum candidate for vaginal drug delivery. Electrochemistry impedance spectroscopy (EIS) was utilized to probe the hole structure in optimum hydrogel immersing in the vaginal fluid stimulant (VFS) for different time. The drug releasing rate of ethinyloestradiol loaded by the optimum hydrogel was examined. The drug releasing results suggested that the releasing rate satisfied with the Higuchi model, while the EIS results confirmed more pores in the composite gels would be formed during the immersing process in the VFS. The results indicated that the gel with the optimum formula met the requirements of vaginal medication. This research suggested poloxamer/chitosan might be an effective type of thermal gel for drug delivery in vagina.

1. Introduction

Mucosal drug delivery system can provide local drug action on the mucosal surface of the body[1, 2]. The vaginal route is commonly used for local delivery of many kinds of the antibacterials and sexual hormones, which can avoid the hepatic first-pass metabolism [3–5]. Physiological parameters should be considered during the design and preclinical evaluation of vaginal formulations. The human vaginal discharge is a complex mixture of cervical mucus, exfoliating epithelia cells, secretions of the Bartholin’s and Skene’s glands, endometrial and fallopian tube fluids. The vaginal pH is in the range from 3.8 to 4.5 for a healthy female [6]. The pH and volume of the vaginal fluid are influenced by the menstrual cycle, age and sexual arousal. The presence of the vaginal fluid would influence the absorption of poorly soluble drugs and reduce residence time on the vaginal mucosa [7]. Therefore, most conventional vaginal formulations usually reside for a relatively short period of time due to the self-cleaning of vagina. The hydrogel would be a good candidate for the drug delivery [8–11]. In this case, many novel delivery systems, such as thermal sensitive gels, films, mucoadhesive solid, etc., have been evaluated for vaginal drug [11–14]. An ideal vaginal drug carrier should be good biocompatibility, less irritating, sustained drug releasing, and biodegradable. However, the commercial drug deliveries haven’t met all the requirements. It is necessary to develop novel drug delivery in vagina.

The thermal sensitive gelling systems are polymeric solutions that undergo sol-gel transitions in response to temperature changes. The gelling occurs in response to the temperature increase from ambient to physiological temperature. In the vaginal administration case, the features of this system should be easy to spread on the mucosa and favoring long permanence of the loaded drug on the mucosa.

Poloxamers are a kind of polymers that exhibit thermal sensitive behavior, with a finely tunable sol-gel transition temperature. Thus they are used for several pharmaceutical and biomedical applications [15, 16]. Chitosan (CS) is the only natural polycationic polymer accounting for its innate mucoadhesive property [17]. Moreover, it possesses valuable properties such as biodegradability, biocompatibility, and capacity for high drug
loading. Numerous CS-based mucosal deliveries have been reported [18]. CS can be modified or combined with other molecules to form injectable thermal sensitive and thermal responsive hydrogels.

In this paper, a series of poloxamer/CS hydrogel compounds were prepared, and the sol-gel transition temperature and rheological property were tested. The hole structure and drug releasing of the optimized hydrogel immersing in vaginal fluid stimulant were evaluated.

2. Materials and methods

Chitosan (CS), glycerol and glacial acetic acid were purchased from Aladdin. Poloxamer 407 (P407) and poloxamer188 (P188) were purchased from BASF, Germany. All the other chemicals were purchased from Sinopharm, China in AR grade without further purification. The deionized water (resistivity of 18 MΩ·cm) was used to prepare the solutions.

The poloxamer/chitosan hydrogel were prepared as the following method. Firstly, CS was dispersed in 0.2 wt% glacial acetic acid aqueous solution with the help of magnetic stirring. Secondly, P407 and P188 were added in the obtained CS solution, and the solution was stirred for 0.5 h. Thirdly, the saturated disodium hydrogen phosphate solution was added in the solution till the pH value arriving 4.2. The obtained solution was stored in fridge at 4 °C for degassing. The solution was placed in water bath, and the increase the bath temperature at the rate of 0.5 °C min⁻¹ up to 37.8 °C. The composition contents of the hydrogels are as the table 1.

Vaginal fluid stimulant (VFS) was prepared as the previous literature [19]. 0.5 ml VFS was added into 5 ml optimized hydrogel. The viscosities of the diluted hydrogel and the as prepared one were measured by rotating viscometer (NDJ-4, Shanghai Changji). The spin speed of the rotor is 60 r min⁻¹, while the temperature increased from 20 °C to 50 °C at the rate of 0.2 °C min⁻¹.

Electrochemistry impedance spectroscopy (EIS) was applied to evaluate the degradation of the hydrogel in VFS. The optimum hydrogel was casted on the glass to form a film. And then the films were immersed in water bath oscillator filled of VFS at 37 °C. The samples were extracted after immersion in the solution for 2 h, 4 h and 6 h. Then the samples were rinsed with deionized water for three times, and soaked in deionized water for 4 h to remove the residual chemicals. The obtained film was sandwiched between two half-cells containing electrolyte solution as the literatures [20, 21]. The EIS data were collected by an electrochemical workstation (CS310H, Corrtest, China). The electrolyte solution was 0.1 mol l⁻¹ KCl aqueous solution. The impedance spectra were taken in the frequency range from 10⁷ Hz to 0.1 Hz. Tha amplitude of the sinusoidal voltage signal was 5 mV.

Ethinyloestradiol (EE) was used as the testing drug. EE was added in the optimized P407/188/CS solution at the content of 2 g l⁻¹. The obtained solution was stored in fridge at 4 °C for degassing. And then the solution was placed in water bath. The temperature increased to 37.5 °C at the rate of 1 °C min⁻¹. The obtained sample was labeled as EE-hydrogel. EE-hydrogel of 0.5 g was placed in a constant temperature water bath oscillator at 37 °C for 5 min to achieve complete gelation. The oscillating rate is 0.8 Hz. 10 ml VFS was added in the gel. Sampling

Table 1. The composition contents of the hydrogel solution.

| CS [wt%] | 0.2 wt% glacial acetic acid solution [wt%] | P407 [wt%] | P188 [wt%] |
|---------|---------------------------------|-----------|-----------|
| 0.2     | 99.8                            | 0         | 0         |
| 0.6     | 99.4                            | 0         | 0         |
| 1.0     | 99                             | 0         | 0         |
| 1.4     | 98.6                            | 0         | 0         |
| 1.8     | 98.2                            | 0         | 0         |
| 2.0     | 81.84                           | 15.7      | 1.46      |
| 1.0     | 74.73                           | 15.73     | 8.54      |
| 1.0     | 79.66                           | 16.15     | 3.19      |
| 1.0     | 77                              | 16.3      | 5.7       |
| 1.0     | 76.85                           | 16.5      | 5.65      |
| 1.0     | 81.5                            | 17.5      | 10        |
| 1.0     | 71.5                            | 17.5      | 5         |
| 1.0     | 71.5                            | 17.5      | 10        |
| 1.0     | 71.19                           | 19.27     | 8.54      |
| 1.0     | 78.24                           | 19.3      | 1.46      |
| 1.0     | 74                              | 20        | 5         |
| 1.0     | 81.84                           | 15.7      | 1.46      |

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followed by replenishment of the same volume of VFS. High performance liquid chromatography (Agilent 1100) was employed to evaluate the drug releasing rate.

3. Results and discussion

The variation of sol-gel transition temperature as a function of the P147 content and P188 content was shown in the figure 1. If P188 content was fixed, the sol-gel transition temperature would decrease when increasing P407 content. If P407 content was fixed, the transition temperature would increase first and then decrease when increasing P188 content. The optimum hydrogels can be obtained at the red region in the figure 1. And the properties of these selected hydrogels were listed in table 2. Therefore, the optimum composition can be selected as P407: P188 = 16.3:5.7.

The effect of CS concentration on the gelling behavior was listed in the table 3. It can be seen that with the increase of CS concentration, the sol-gel transition temperature decreases, and the viscosity and pH value increase. For a healthy woman, the temperature in vagina is in the range from 37.2 °C to 37.8 °C [22]. The designed thermal sensitive hydrogel should turn to gel at the temperature slightly lower than body temperature. In this case, the expected sol-gel transition temperature should be around 32 °C to 36 °C. Considering the viscosity, sol-gel transition temperature and pH value, CS concentration of 1 wt% was chosen for the next step.

Due to the presence of the vaginal fluid, it is necessary to investigate the effect of vaginal fluid on the gelling temperature. The presence of the vaginal fluid may vary the viscosity of hydrogel. In this case, the viscosities of the as prepared hydrogel and hydrogel diluted by VFS were examined, and the results were shown in figure 2. It
can be found that the viscosity of the diluted hydrogel slightly decreases, and the gelling temperature increases up to 36 °C, which confirmed that this hydrogel can be applied for the vaginal drug.

In order to further study the variation of the pores in hydrogel, the poloxamer/CS hydrogel after gelling was immersed in VFS at different time, and then probed by EIS. The EIS results were shown in figures 3 and 4. It can be clearly found that each spectrum exhibits a semicircle and a tilted spike in Nyquist plot. Besides, the semicircle became smaller with increasing the immersing time. The semicircle is related to the charge permeation through the film, while the spike is associated to the electrode/solution interface [23]. Thus the equivalent circuit for this system could be consisted of the following sections: (1) solution resistance, (2) parallel association of the film resistance for charge permeating through the pores and capacitance for the bulk film, (3) Warburg impedance element. According to the equivalent circuit, the impedance can be expressed as

\[
Z(\omega) = R_s + \frac{R_m}{1 + (\omega R_m C)^2} - j\omega C R_m^2 / [1 + (\omega R_m C)^2]
\]  

(1)

\[
|Z| = \sqrt{\left[R_s + R_m/[1 + (\omega R_m C)^2]\right]^2 + \left[\omega C R_m^2/[1 + (\omega R_m C)^2]\right]^2}
\]  

(2)

where \(R_s\) refers to the resistance of the electrolyte solution, while \(R_m\) and \(C\) are associated to the resistance and capacitance of the hydrogel film, respectively.

In figure 4, the Bode plots exhibit two plateaus at different simulated frequencies. According to equation (2), it can be easily to confirm that the lower plateau at the high frequencies refers to \(R_s\) while a higher plateau at low frequencies associated to \(R_s + R_m\). Consequently, it can be concluded that the film resistance decreases with
increasing the immersing time. The film resistance is related to the tunnels in the film for ion permeation. In other words, for the present system, the charge permeation was determined by the pore structure in the film. Besides, the Nyquist plots indicated the semicircle became smaller with increasing the immersing time. And smaller semicircle implied easier charge permeation through the film [23]. Therefore, EIS results suggested that more and/or larger pores for charge permeation were formed during the immersing process. In other words, the immersing in VFS leads to the degradation of hydrogel, which favor the drug releasing through the hydrogel.

EE has been widely used in the treatment of menopausal symptoms, in functional uterine bleeding, for inhibition of lactation, and also for palliative treatment of breast cancer in postmenopausal women and prostate cancer [24, 25]. The drug releasing rate was shown in figure 5. According to the EIS results, more pores would be formed in the hydrogel during the immersing process. These pores act as the tunnels for the drug molecules permeation. In the present study, the drug molecules transport through the hydrogel should obey the Fick’s diffusion law, and the drug dissolution is rapid and a large excess of drug is provided. Thus, the releasing rate may fulfill the Higuchi equation [26, 27]. In this case, the fitting curve was obtained with Higuchi model according to the experimental data. The releasing rate can be expressed as the following formula:

\[
y = 7.6 \times t^{1/2} + 0.1
\]

Therefore, the equation (3) confirmed that the releasing rate of EE loaded on the poloxamer/CS hydrogel can well satisfy with the Higuchi model.
4. Conclusions

A series of poloxamer-CS hydrogels were prepared. An optimum hydrogel with the composition that CS: P407: P408 = 1: 16.3: 5.7 was obtained. The dilution experimental results confirmed that gelling temperature and viscosity varied slightly when the hydrogel diluted by VFS, suggesting the hydrogel could be applied in vagina. EIS results indicated that more and/or larger pores were formed in the hydrogel during the immersing process, implying the degradation of hydrogel during the application. The releasing rate of EE loaded by the hydrogel indicated the releasing phenomena were consistent with the Higuchi model.

Conflicts of Interest

The authors declare no conflict of interest.

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